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14. ABSTRACT

The Integrative Cardiac Health Project (ICHP) aims to lead the way in Cardiovascular Disease (CVD) Prevention by conducting novel research utilizing a Systems Biology / personalized medicine design to discover and develop practical, effective and preemptive integrative approaches in order to detect and combat CVD earlier before it affects the quality of life. ICHP's ultimate goal is to translate our evidenced-based research findings for application into clinical practice. A translational research approach will provide the ability to find novel disease markers, optimal prevention and holistic treatment approaches, and a unique venue for future research as the "virtual laboratory" for optimal comprehensive health prevention in the military beneficiary population. This research method also allow us to further hypothesize and define relationships between CVD, other cardio metabolic disease states and maladaptive behavior patterns unique to service members such as pre-diabetes, stress, overweight and sleep disorders with the aim of targeting these disorders in a pre-clinical phase. Using an integrative, interdisciplinary preventive health approach, ICHP has shown that an individual's cluster of CV risk factors can be effectively targeted and improved.

15. SUBJECT TERMS
Lifestyle; Cardiac Prevention; Behavior; Coronary Heart Disease (CHD); Proteomics; Genomics; Metabolic Syndrome; Cardiovascular Disease (CVD); Diabetes; Obesity; Stress

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Executive Summary - Integrative Cardiac Health Project
Annual Report FY09 Yr 1
Dates: 20 Aug 2010 - 19 Aug 2011

The Integrative Cardiac Health Project (ICHP) aims to lead the way in Cardiovascular Disease (CVD) Prevention by conducting novel research utilizing a Systems Biology / personalized medicine design to discover and develop practical, effective and preemptive integrative approaches in order to detect and combat CVD earlier before it affects the quality of life. ICHP's ultimate goal is to translate our evidenced-based research findings for application into clinical practice. In keeping with this aim, collaborative research efforts have continued between ICHP projects at Walter Reed Army Medical Center, Windber Research Institute and Windber Medical Center. In the past quarter, the following key accomplishments are noted:

- Total yearly visits at ICHP WRAMC/Windber Prevention Programs: 3635 (Past quarter = 826 visits. Visits include telephonic follow-up)
- Protocols completed -1; 5 protocols (including 1 sub study) in data analysis phase
- 3 active protocols in progress; planned submission of 2 new protocols next quarter
- Dissemination of scientific research findings continues:
  - 2 manuscript published; 5 in preparation
  - 3 abstracts presented as podium presentations at national meetings
  - 13 abstracts presented as posters at national meetings (1 abstract selected as the 1st place winning abstract); 2 abstracts accepted
- Intense planning/preparation for relocation to WRNMMC Bethesda on 29 August 11
- Data analysis continues. Relevant findings in our population include during the past quarter:
  - BATTLE Study findings suggest knowledge of an abnormal CIMT and increased CV risk does not improve adherence to a lifestyle program
  - Molecular analysis continues on the Ornish Program participants – changes in gene expression occur at 12 weeks and persist at one year:
    --Genes involve function in defense and immune response
    --Non-diabetics with high insulin show greater gene expression changes than diabetics or non-diabetics with low insulin
    --Participants with low stress show more expression changes than high-stress participants
  - In several studies of gene expression changes, the following was observed:
    --Gene expression changes restricted to non-diabetics with high insulin compared to diabetics and non-diabetics with low insulin
    --Most expression changes occur in low-stress versus high-stress group
  - Genetic variation influences risk factor response
    --16 SNPs showed evidence of an influence on triglyceride response
    --3 SNPs showed differences between genotype groups
    --4 SNPs showed differences by gender for one genotype
  - There is evidence that important differences in levels of perceived stress, sleep quality and daytime sleepiness between white and black subjects in our program exist. These differences deserve explanation and may be valuable in designing interventions tailored for specific groups.
- Clinical Transition Strategy Plan for facilitating a smooth transition and maintaining high quality standards of our DOD COE at WRNMMC to serve all military beneficiaries, including website creation.
- Significant innovations in Clinical Flow Plan of CPP, refinement of processes; creation of new clinical positions to support expanding research initiatives.
- Identified need for specific track for pre-diabetes and diabetes care; development of pre-diabetes and diabetes clinical track using novel team approach of integrative care.
- Continued ICHP Database and Platform Creation: Functional requirements discussed with HJF. Awaiting vendor selection. Data variables outlined and streamlined for database creation.

Introduction

The primary vision of the ***Integrative Cardiac Health Project (ICHP)*** is to lead the way in Cardiovascular Disease (CVD) Prevention by conducting novel research utilizing a Systems Biology / personalized medicine design to discover and develop practical, effective and preemptive integrative approaches in order to detect and combat CVD earlier before it affects the quality of life. ICHP's ultimate goal is to translate our evidenced-based research findings for application into clinical practice in an effort to achieve the following research aims:

- ❑ Improve Force Health by better understanding the CVD risk susceptibility of military specific populations as well as to understand the individual service member through leading-edge research using novel tools and technologies. Synchronize our programs with existing DOD efforts in Comprehensive Soldier Fitness (CSF), Comprehensive Behavioral Health System of Care (CBHSOC) and the Warrior Resiliency Program.
- ❑ Investigate and create transformational models of healthcare delivery through personalized CVD prevention tracks as an adjunct to traditional care.
- ❑ Refine individualized prevention strategies through statistical data modeling to define the most cost-effective and sustainable approaches in promoting cardiovascular health throughout the military lifecycle.
- ❑ Simultaneously, improve understanding of the molecular, physiological, biochemical, immunological and environmental basis of CV health and disease and to use that understanding to develop improved approaches to disease diagnosis, treatment and prevention, in line with NHLBI Strategic Plan 2008.

Body

Completed tasks or tasks removed from SOW during this reporting period are not reflected in the Gantt charts submitted with this report.

Task #1: "Non-Invasive Coronary Artery Disease Reversal" (CADRe) Study Protocol.

Status: Task complete. This task will be removed from subsequent quarterly reports. The following final manuscript was published (see Appendix A):

- Marshall D, Walizer E, & Vernalis M. Effect of a One-Year Lifestyle Intervention Program on Carotid Intima Media Thickness, *Mili Med* 2011; 176(7): 798-804.

Task #3: Ongoing data collection for "CADRe Five-Year Follow-up" Protocol.

Status: WRAMC HUC approved this study on 23 May 2006. Protocol was approved by the US Army Medical Department Center and School, Clinical Investigations Regulatory Office (CIRO) on 2 November 06. The MRMC Memorandum of Deferral was received 22 Jan 07. Change of PI to COL Randolph Modlin, Chief, WRAMC Cardiology, was approved 3 December 2008. Study addendums have been previously reported. The annual Continuing Review was approved by WRAMC DCI HUC on 19 April 2011 and has been forwarded to MRMC for review. This study is closed to accural, data collection is complete and final data analysis is in progress. Publication plan in progress.

Study Design and Objectives

This follow-up study will determine the persistence of healthy lifestyle behavioral changes and CVD risk factor control results after their original CADRe study participation. This study will continue as a longitudinal observational study where patients will have yearly follow-up visits at 1, 2, 3, 4, and 5 years after completion or expected completion of the CADRe Study. This study will involve prospective collection of data, however, there will be no tests ordered that are not considered WRAMC Cardiology standard of care for the study population identified. Therefore, there are no risks involved with this study outside those of the standard of care treatment. Specific aims are to determine:

1. Persistence of lifestyle change behaviors in diet, exercise, and stress management
2. Coronary risk-factor control
3. Quality of Life

Hypothesis

Subjects who have been exposed to an intensive lifestyle change program will demonstrate long-term carryover of heart healthy characteristics including persistence of favorable lifestyle change behaviors and risk factor control.

Recruitment/Enrollment

Up to 163 male and female CADRe study participants, age 18 years or older, with subsequent completion of Phase 1 of the CADRe Study (3-month data collection) were recontacted and invited to participate in this 5-year follow-up study (post-study completion or expected completion).

Outcome Measures

A composite index of 7 heart healthy characteristics (BMI 18.5 – 25; LDL-cholesterol < 100 mg/dL; dietary fiber intake ≥ 25 gms/day; consumption of 5 or more fruits and vegetables per day; BP < 140/90 mmHg; regular exercise ≥ 150 min/week, and daily practice of CADRe program stress management techniques) was selected as the primary outcome measure since the main goal of this study is to assess the persistence of lifestyle change behaviors and risk factor control. The Heart Health Index (HHI), presented as a single score (range 0-7), will be assigned to each subject yearly. Additionally, each of the 7 heart healthy characteristics will be assessed independently as a continuous variable. Secondary outcome measures include: Changes in modifiable CVD risk factors (blood pressure, body composition and fitness, lipid levels and glucose); C-reactive protein and, Quality of Life.

Preliminary Findings:

Of the 163 eligible, 102 participants responded (63%) to the study mailing: 80 meet eligibility criteria and agreed to make a study visit; 2 were ineligible; 17 declined screening interview / participation; 2 were undecided about participation, and; 1 deceased. Of the 80 eligible who agreed to make a study visit, 76 provided informed consent for at least 1 follow-up visit. Fifty-one participants provided at least 1 additional follow-up study visit. See Table 1 for actual longitudinal follow-up of study participants by cohort.

Table 1. Actual Longitudinal Follow-Up of CADRe Study

				Actual Visits					
Cohort	# Pts Available	# Replied	# Enrolled	Year 1	Year 2	Year 3	Year 4	Year 5	Total Visits
1	7	3	3					3	3
2	15	6	3					3	3
3	14	7	7					7	7
4	17	10	9				6	8	14
5	12	8	7				5	7	12
6	19	12	6				4	6	10
7	15	9	5			4	3	4	11
8	12	6	4			4	4	4	12
9	9	5	5			4	4	2	10
10	10	9	9		7	8	6	6	27
11	11	5	5		5	5	4	3	17
12	10	3	3		3	3	1	3	10
13	12	10	10	9	9	10	8	8	44
Totals	163	90	76	9	24	38	45	64	180

At study enrollment, participants were 66 yrs old (range 36 to 80), predominantly Caucasian male (79%) and significantly overweight with a BMI of 29.8; similar to their pre-CADRe study BMI of 29.1. Subjects were a mean of 3.2 yrs post CADRe Study completion or anticipated completion. Of the individual CADRe Study lifestyle components, participants were most compliant with exercise (goal ≥ 180 minutes/week): mean weekly time =183 minutes of moderate to vigorous physical activity. Although few participants reported a strict vegan dietary pattern following completion of the CADRe Study, dietary fiber and average fruit and vegetable intake was higher than the average U.S intake at 29 grams/day and 9.7 servings per day, respectively. Participants continued to have difficulty in performing 1 hour of stress management daily. Participants reported an average time of 154 minutes/week spent in any of the five CADRe Study techniques with only 33% reporting daily performance of at least 1 stress management technique. Table 2 provides a preliminary descriptive analysis for the major outcome variables as an aggregate sample for the initial study visit. Individual HHI scores (composite score of heart healthy behaviors) has not yet been calculated, however, at least 5 of the 7 heart healthy behaviors are being met in the aggregate (LDL-cholesterol < 100 mg/dL; dietary fiber intake ≥ 25 gms/day; consumption of 5 or more fruits and vegetables per day; BP < 140/90 mmHg, and regular exercise ≥ 150 min/week).

Table 2: Major Outcomes Variables at Initial Study Visit

Outcome Variable (n=76)	Mean	SD
Weight (kg)	88.4	22.5
Body Mass Index (BMI)	29.8	6.3
% Body Fat (n=74)	31.2	9.8
Systolic BP (mmHg)	125.6	12.6
Diastolic BP (mmHg)	71.3	6.8
Fasting Glucose (mg/dL)	94.8	13.4
Total Cholesterol (mg/dL)	154.4	35.5
HDL Cholesterol (mg/dL)	50.2	11.4
LDL Cholesterol (mg/dL)	86.2	30.7
Triglycerides (mg/dL)	129.6	67.8
C-Reactive Protein (mg/dL) (n=74)*	0.197	0.365
Daily Dietary Fiber (gms)	28.6	14.1
Daily Fruit/Vegetable Servings	9.7	5.5
Weekly Exercise Time (minutes)	183.0	167.0
Weekly Stress Mgt Time (minutes)	154.1	166.8
Physical Composite Score	44.2	11.5
Mental Composite Score	54.1	8.2

*Two outliers (C - reactive protein > 6.00) excluded from analysis

Preliminary analysis of changes in modifiable CVD risk factors (BP, body composition and fitness, lipid levels and glucose) can be assessed in comparison to the final CADRe Study visit (Table 3) in those subjects who have completed a 5-year follow-up study visit. In 62 participants with Year 5 data, there were significant increases in both body composition and systolic BP when compared to final CADRe study visit data. Body anthropometrics show an 8% mean weight gain and a 22% increase in body fat despite reporting a mean of 175 minutes per week of moderate physical activity. Systolic BP increased by 4%. No significant change was seen in diastolic BP, glucose, HDL or CRP. However, significant reductions in TC, LDL and TG at Year 5 were 4%, 4% and 10%, respectively. Of the 56 participants on lipid-lowering medications, 93% reported either no change (n=29) or an increase (n=23) in these medications which may account for the lipid profile changes.

Table 3. Select Outcome Variables at 5-Year vs. Final CADRe Study Visit (n=62)

	Final CADRe Study Visit	5-Yr Follow-up Visit	Change	P
Body Composition/Blood Pressure (BP)				
Weight (kg)	82.5 ± 22.6	89.2 ± 25.3	6.7 ± 9.9	<0.001
BMI (kg/m^2)	27.6 ± 6.1	30.2 ± 6.7	2.6 ± 3.6	<0.001
% Body Fat*	26.4 ± 9.1	31.2 ± 9.2	4.8 ± 4.3	<0.001
Systolic BP (mmHg)	120.8 ± 12.4	125.4 ± 14.7	4.6 ± 14.2	0.014
Diastolic BP (mmHg)	69.1 ± 7.3	70.9 ± 7.7	1.8 ± 8.9	0.121
Laboratory (mg/dL)				
Glucose (mg/dL)**	96.8 ± 15.7	96.2 ± 17.9	-0.7 ± 16.5	0.746
Total Cholesterol (mg/dL)	158.9 ± 31.3	150.3 ± 30.1	-8.6 ± 29.9	0.027
LDL-Cholesterol(mg/dL)	87.1 ± 23.2	81.5 ± 24.5	-5.6 ± 21.6	0.046
HDL-Cholesterol(mg/dL)	46.1 ± 10.2	48.7 ± 13.1	2.6 ± 11.2	0.076
Triglycerides (mg/dL)	157.7 ± 88.8	130.0 ± 84.5	-27.8 ± 75.5	<0.001
C-reactive protein (mg/dL)#	0.226 ± 0.275	0.242 ± 0.412	0.016 ± 0.379	0.479

Values are mean ± SD; *n=57; **n=61; #n=60.

Adverse Events : There have been 2 adverse events (AEs) reported to the WRAMC HUC during the course of this study and previously reported.

Task #4: Ongoing enrollment for "*B*etter Adherence *to T*herapeutic *L*ifestyle Change *E*fforts (BATTLE) Trial".

Status: WRAMC HUC approved protocol on 25 April 2006. Final study approval from CIRO was granted on 28 April 2006. The MRMC Memorandum of Deferral was received 5 Jul 06. Change of PI to COL Randolph Modlin, Chief, WRAMC Cardiology, was approved 3 December 2008. Six approved study addendums have been previously reported. The annual Continuing Review was approved by WRAMC DCI HUC on 5 November 2010 and forwarded to MRMC. This study is closed to accrual, data collection is complete. Main study database hard locked in June and draft data tables received mid-July. Review of data tables in progress. Data analysis also in progress for formative evaluation of TLC intervention. Publication plan in progress. "Methods/Demographics" manuscript and "Integration of mindfulness into a support group curriculum" in progress.

The following abstract was *presented* as a poster at a national meeting (See Appendix A):

- Saum N, Walizer E, Vernalis, M. Feasibility of including limited mindfulness training in an existing therapeutic lifestyle change (TLC) program. Prevention Cardiovascular Nurses Association (PCNA), Mar 2011: Orlando, FL.

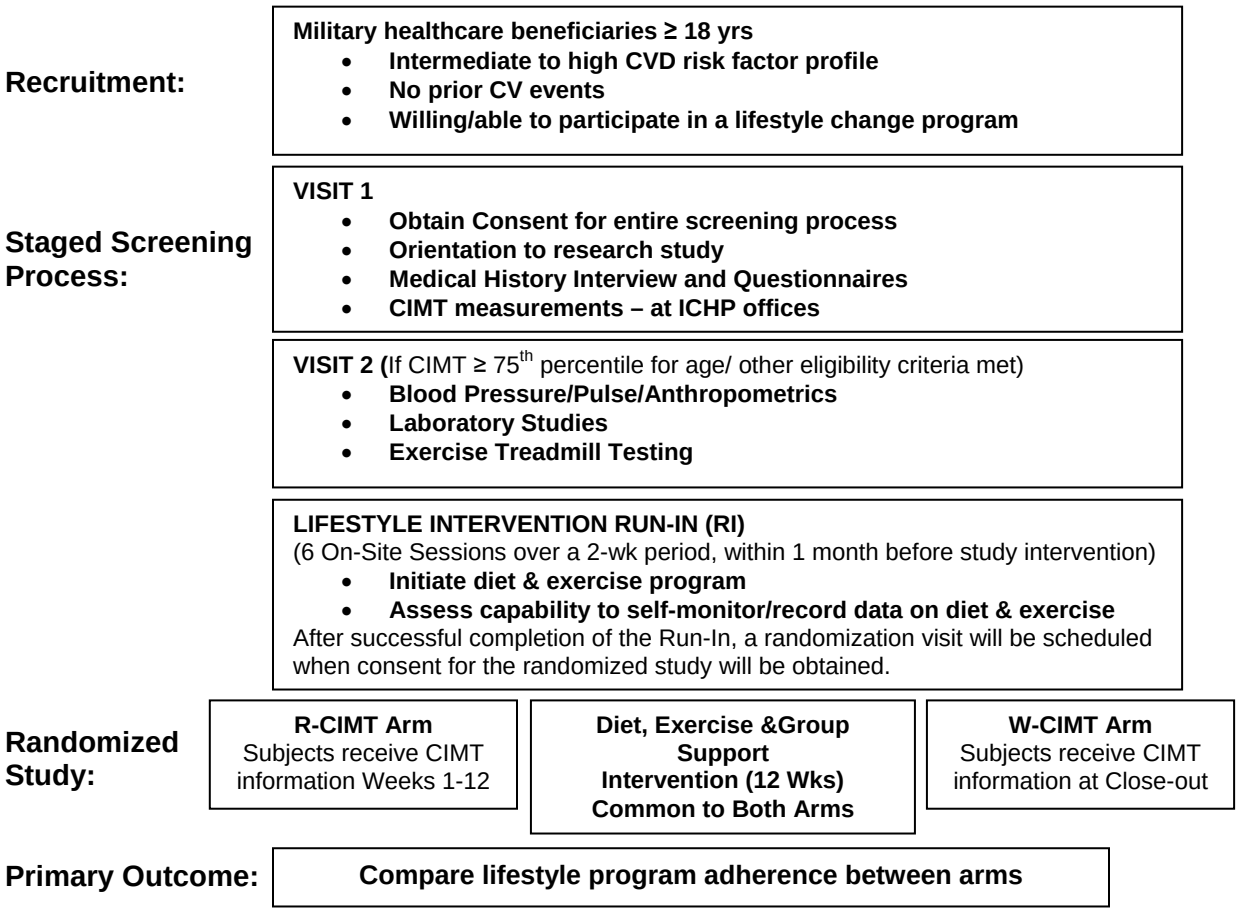
The following manuscript is in preparation. Planned submission next quarter:

- Saum NS, Halsey JF, Walizer EM, Vernalis MN. Exploring the role and impact of limited mindfulness training in changing diet and exercise behaviors. (In preparation).

Study Design and Objectives

The purpose of this study is to determine whether knowledge of abnormal results from a noninvasive test for detection of subclinical atherosclerosis (CIMT), in addition to knowledge of CVD risk factors, enhances adherence to healthy lifestyle behaviors in comparison to only CVD risk factor knowledge. This two-arm, double-blinded study (see Figure 1) will randomize subjects to either receive CIMT results (R-CIMT Group) or have CIMT results withheld (W-CIMT Group) in the setting of a 3-month TLC intervention. After the 3-month TLC intervention period is completed, subjects who had CIMT results withheld will receive this information. Because knowledge of the study hypothesis could impact their behavior during the lifestyle intervention, subjects will be blinded to the study hypothesis. Similarly, research staff implementing the TLC intervention will be blinded to subjects' randomization assignment. Randomization assignment will also be blinded in the assessment of endpoints.

Figure 1. BATTLE Trial Design



Hypothesis

Subjects with CVD risk factors who have knowledge of their own CIMT test results showing significant subclinical atherosclerosis will demonstrate better adherence to TLC than those subjects from whom the CIMT test information is withheld.

Primary Outcome Measure

A composite index of adherence to the TLC intervention was selected as the primary outcome measure since the main goal of this study is to assess the impact of CIMT imaging knowledge on change in lifestyle behaviors. A combined measure of adherence, reflecting both aspects of the lifestyle intervention (Mediterranean-type diet, moderate aerobic exercise), was chosen that uses accepted measures of diet and exercise adherence reported in the literature. Although any of the modifiable CVD risk factors could have been selected as surrogate markers of adherence, factors aside from behavioral change could affect changes in these risk factors such as individual variability in response to the interventions or changes in pharmacologic therapy by the subjects own healthcare providers who are separate from the research staff. Also, not all subjects are expected to need improvement in the same CVD risk factor, thus, selection of a single risk factor as the primary outcome variable would be difficult and arbitrary. Use of a composite measure of risk factors, such as the Framingham Risk Score, is not validated for serial assessment in a short-term study.

Secondary Outcomes

Several additional outcomes will be assessed including:

- Adherence to each TLC Program component: Diet, Exercise, Attendance at weekly on-site sessions
- Changes in modifiable CVD risk factors: blood pressure, body composition and

fitness, lipid levels, glucose/insulin resistance
- Other biochemical markers: C-reactive protein (CRP)
- Emotional factors: Anxiety Score, Stage of Change related to lifestyle behaviors, Self-efficacy, Motivation
- Atherosclerosis and CIMT Knowledge Assessment Score (only in CIMT-R subjects)

Study Population

The study will be conducted with individuals at moderate to high risk for cardiovascular events based on CVD risk factor profile and evidence of significant subclinical atherosclerosis. From our previous experience in recruiting military healthcare beneficiaries for a lifestyle intervention study, it is projected that one-third of the population screened will meet CIMT criteria > 75th percentile for age. Despite this high ratio of screened to eligible subjects, the presence of an abnormal scan is a crucial feature in the study design, which will specifically allow the motivational impact of CIMT imaging to be determined.

Preliminary Findings:

Since November 2007, 1068 patients have given permission for a study team member to contact them regarding this study. Approximately 41% of "interested" patients telephonically screened were eligible to initiate the study screening process. Over 48% of those contacted opted out (n=507) of the study primarily for time commitment and travel/distance reasons. The primary reason for ineligibility on the initial telephone screen was low cardiovascular risk profile. Of the 275 consented subjects who were considered screen failures, 60% screened out primarily by CIMT (<75 percentile for gender/age), 14% had an acceptable past medical history, 11% withdrew consent, 6% did not meet diagnostic or severity criteria, 1% had an intercurrent medical event and 8% were categorized as other (deployment, relocation, job conflicts). In summary, approximately 18% of those patients who met initial screening criteria after the telephone screen (n=948) randomized into the main study. See Table 4 for study enrollment and disposition by group (received CIMT [R-CIMT] vs. control [W-CIMT) and total sample.

Table 4. Subject Enrollment and Study Disposition

Parameter Category	R-CIMT	W-CIMT	Total
Initial Telephone Screen Completed			1068
Subjects Screened			441
Subjects Randomized	83	83	166
Subjects Included in Evaluable Population	69 (83.1%)	73 (88.0%)	142 (85.5%)
Subjects with Study Completion	69 (83.1%)	73 (88.0%)	142 (85.5%)
Subjects Who Terminated Early from the Study	14 (16.9%)	10 (12.0%)	24 (14.5%)
Primary Reason for Early Termination:			
-- Ineligibility	0	0	0
-- Adverse Event	2 (2.4%)	2 (2.4%)	4 (2.4%)
-- Subject Withdrew Consent	4 (4.8%)	3 (3.6%)	7 (4.2%)
-- Investigator Judgment	0	0	0
-- Protocol Non-compliance (After Randomization)	2 (2.4%)	3 (3.6%)	5 (3.0%)
-- Lost to Follow-up	2 (2.4%)	1 (1.2%)	3 (1.8%)
-- Termination of Study by Sponsor	0	0	0
-- Subject Death	0	0	0
-- Other	4 (4.8%)	1 (1.2%)	5 (3.0%)

Thirty RI groups were conducted with 166 subjects successfully completing the RI phase and randomizing into the Main Study. Thirty Main Study Support Groups yielded 142 study completers and 24 non-completers (14.5% dropout rate). The primary reasons for early termination are outlined in Table 4.

Preliminary Findings

During this past quarter, in collaboration with PREMIER Research, data reconciliation and quality control of the data have been completed. The study data base was locked on 21 Jun 11 and in mid-July, PREMIER provided draft data tables for review. Analysis was conducted on both the evaluable subjects (study completers=142) and intent-to-treat subjects (subjects who at least one post randomization visit=161). For this report, we will provide preliminary findings for the completers only.

Study completers were predominately middle aged, overweight (mean BMI=31.5 ± 5.6), Caucasian, females (see Table 5); however, statistical significant differences in the study groups were detected in mean age and gender. The treatment group was older and comprised of more women. No differences between groups was detected in overall reported comorbid conditions, however, over 50% of the women in the treatment group were postmenopausal. The CVD risk profile of completers is as follows: 53% hypertensive, 82% dyslipidemic, 12% Type 2 diabetes, 4% current smokers, and 56% with family history of CVD; no differences were detected between the treatment groups.

Table 5: Demographic Characteristics (Study Completers)

Parameter Category/Statistics	R-CIMT (N=69)	W-CIMT (N=73)	Total (N=142)	p-value
Age (Yr)				0.015[1]
N	69	73	142	
Mean (SD)	56.8 (9.35)	52.6 (10.98)	54.7 (10.40)	
Median	57.0	53.0	55.0	
Min, Max	26, 75	30, 78	26, 78	
Gender				0.018[2]
-- Male	18 (26.1%)	33 (45.2%)	51 (35.9%)	
-- Female	51 (73.9%)	40 (54.8%)	91 (64.1%)	
Race				
-- American-Indian or Alaska Native	3 (4.3%)	1 (1.4%)	4 (2.8%)	
-- Asian	1 (1.4%)	3 (4.1%)	4 (2.8%)	
-- Black or African-American	29 (42.0%)	35 (47.9%)	64 (45.1%)	
-- Native Hawaiian or Other Pacific Islander	0	0	0	
-- White or Caucasian	38 (55.1%)	30 (41.1%)	68 (47.9%)	
-- Other	2 (2.9%)	7 (9.6%)	9 (6.3%)	
Weight (kg)				0.991[1]
N	69	73	142	
Mean (SD)	90.53 (19.318)	90.50 (16.955)	90.51 (18.077)	
Median	87.70	91.50	90.80	
Min, Max	53.1, 127.0	58.4, 145.5	53.1, 145.5	

[1] ANOVA model with CIMT group as the factor; if the assumption of normal distribution is violated then Wilcoxon-Mann Whitney test (non-parametric method) will be applied instead; [2] Chi-square or Fisher's exact test as appropriate.

A composite index of adherence to the TLC intervention was selected as the primary outcome measure since the main goal of this study is to assess the impact of CIMT imaging knowledge on change in lifestyle behaviors. A combined measure of adherence, reflecting both aspects of the lifestyle intervention, was chosen that uses accepted measures of diet and exercise adherence reported in the literature. At study closeout, both groups showed marked improvement in both diet and exercise adherence as compared to baseline, however, no difference was detected between the study groups, thereby, confirming the null hypothesis that

knowledge of an abnormal CIMT scan did not have a motivational impact on overall adherence to the TLC intervention in this study (see Table 6).

Table 6: Primary Efficacy Analysis: Mean Adherence Change

Parameter	Statistics	R-CIMT (N=69)	W-CIMT (N=73)	Total (N=142)	p-value
Adherence at Baseline	N	69	73	142	
	Mean (SD)	45.447 (16.6740)	40.678 (17.2143)	42.995 (17.0623)	
	Median	44.762	39.722	41.111	
	Min, Max	12.14, 82.14	7.14, 78.57	7.14, 82.14	
	Q1, Q3	32.103, 60.357	27.937, 51.786	30.357, 57.143	
Adherence at Week 12 OC*	N	69	73	142	
	Mean (SD)	65.060 (20.1362)	63.322 (22.0451)	64.166 (21.0824)	
	Median	62.063	66.746	63.075	
	Min, Max	28.57, 97.78	25.00, 100.00	25.00, 100.00	
	Q1, Q3	51.190, 82.143	42.421, 85.714	45.754, 83.929	
Adherence Change (%)	N	69	73	142	**0.519**[1]
	Mean (SD)	19.613 (24.3395)	22.644 (24.2286)	21.171 (24.2440)	
	Median	16.746	24.365	21.429	
	Min, Max	-39.29, 73.81	-50.00, 72.26	-50.00, 73.81	
	Q1, Q3	3.452, 36.270	4.206, 41.627	3.532, 38.889	

Note: P value of change from baseline is obtained by ANCOVA model with CIMT group, gender as factors and age as the covariate; if assumptions are invalid, non-parametric ANCOVA will be applied

[1] indicates that P value is obtained from parametric method;

[2] shows P value is obtained from non-parametric method.

* OC refers to the measurement observed cases at week 12. Adherence will be calculated by OC of Mediterranean Diet % adherence and OC of Exercise % adherence; adherence is capped at 100%

Although the hypothesis was not supported, study completers did make significant improvements in most of their modifiable risk factors (anthropometrics; total and LDL-cholesterol; triglycerides. Slight increases were seen in systolic and diastolic blood pressure.

Data analysis on these completers, comparing study completion to baseline, was conducted using *t-test* and *Wilcoxon Sign Test* statistics (see Table 7). Measures of obesity including weight, BMI and % body fat were reduced by 5%. Additionally, a 5% reduction in waist circumference and a 7% reduction in abdominal sagittal diameter were seen. Both systolic and diastolic blood pressure increased by 2%.

Levels of total cholesterol were reduced by 6%, LDL-cholesterol decreased by 9% and triglycerides were lowered by 14%. C-reactive protein (CRP) was decreased by 17%. Despite these positive changes, a 1% reduction in HDL-cholesterol was seen.

Serum fasting glucose and insulin were collected and HOMA scores calculated as a measure of insulin resistance (IR). At baseline, 48% of the study completers had HOMA score > 2.8, indicative of IR. At study completion, 19 subjects were able to lower their HOMA scores < 2.8 and reduce their risk of pre-diabetes. Overall, serum glucose was reduced by 4% and fasting insulin was reduced by 23.3%.

Table 7. Secondary Outcome Variables in Study Completers (n=142)

	Baseline	Study Completion	Change	P
Body Composition				
Weight (kg)	90.5 ± 18.1	86.1 ± 17.3	-4.5 ± 3.8	<0.001[1]
BMI (kg/m^2)	31.5 ± 5.6	29.9 ± 5.5	-1.5 ± 1.3	<0.001[1]
% Body Fat	37.1 ± 8.5	35.3 ± 8.7	-1.8 ± 2.6	<0.001[1]
Sagittal Diameter (cm)	24.0 ± 3.8	22.3 ± 3.7	-1.7 ± 1.6	<0.001[1]
Waist Circumference (cm)	100.6 ± 13.1	95.6 ± 13.1	-5.0 ± 4.1	<0.001[1]
Laboratory (mg/dL)				
Glucose (mg/dL)	96.8 ± 19.0	93.1 ± 14.1	-3.7 ± 14.6	0.003[1]
Insulin (uIU/mL)	14.6 ± 12.6	11.2 ± 8.0	-3.4 ± 7.6	<0.001[1]
HOMA (Insulin Resistance)	3.9 ± 4.4	2.8 ± 2.8	-1.1 ± 3.3	<0.001[2]
Total Cholesterol (mg/dL)	195.8 ± 40.1	184.1 ± 35.5	-11.8 ± 26.0	<0.001[1]
LDL-Cholesterol(mg/dL)	119.5 ± 35.5	108.4 ± 28.8	-11.2 ± 22.0	<0.001[1]
HDL-Cholesterol(mg/dL)	56.0 ± 15.9	55.4 ± 14.5	-0.61 ± 7.7	0.348[1]
Triglycerides (mg/dL)	122.2 ± 62.4	105.3 ± 52.6	-16.9 ± 43.8	<0.001[1]
C-reactive protein (mg/dL)	0.425 ± 0.528	0.352 ± 0.461	-0.074 ± 0.318	0.007[2]

Values are mean ± SD;
[1] indicates that P value is obtained from parametric method;
[2] shows P value is obtained from non-parametric method.

Although these data do not support the motivational impact of an abnormal CIMT scan on program adherence, more analysis will be conducted to fully describe the study results. It is clear that this data supports participation in a lifestyle modification program which includes education and frequent monitoring does result in substantial CV risk factor improvements. Some of these changes rival what has been observed with pharmacological treatment.

Preliminary Analysis for Summative Evaluation of Lifestyle Program (Addendum #6):
This formative evaluation took place between Jan-Mar 2011. Of the 140 surveys mailed to consenting BATTLE Study participants, 49% (n=68) were returned. Additionally, 36 completers from study year 2 and 5 non-completers gave permission to be contacted for a telephone interview. Of these 41 participants, 35 telephonic interviews were conducted over this 2 month period. All telephone interviews have been transcribed. Frequency counts of survey responses and thematic coding of telephone interviews and open ended survey responses are in progress.

Protocol Deviation: One protocol deviation was reported to the WRAMC DCI Human Use Committee during this study and previously reported.

Adverse Events: During the course of this study, 10 serious (SAEs) and 25 non-serious AEs have been reported to WRAMC DCI Human Use Committee. A summary of AEs has been previously reported.

Task #5: Transition away from the traditional Dr. Dean Ornish Program for Reversing Heart Disease protocol.

Status: Enrollment into the Dr. Dean Ornish Program is closed and all active participants have completed their participation in the study. Data analysis is ongoing.

Subject Enrollment and Demographics
This program is closed to enrollment and all active subjects have completed the program. Subject enrollment was 422 participants including 25 cohorts and 4 retreats. 339 participants graduated from the program and 83 participants discontinued participation (20% dropout rate). Demographic characteristics of participants were: average age of 66.1 years, 53% female, 33% veterans or the spouse of a veteran, and 41% had diagnosed coronary heart disease.

Outcome Data
Participants in the Dr. Dean Ornish Program at Windber Medical Center achieved significant improvement in levels of virtually all of the measured coronary artery disease (CAD) risk factors over the initial 12-week period. Measures of obesity including weight and BMI declined ~7%, levels of total cholesterol were reduced by nearly 13%, blood pressure dropped ~9%, measures of physical fitness increased more than 26%, and levels of depression decreased approximately 47%. These data demonstrate that lifestyle change programs may be important for primary prevention in individuals with diagnosed CAD and those at increased risk of disease. Over the course of one year, weight and BMI decreased ~9%, diastolic blood pressure decreased ~7%, measures of physical fitness increased 25%, and levels of depression decreased nearly 50%.

Task #6: Complete enrollment in Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal, Sub-Study for Subjects in the Dr. Dean Ornish Program protocol.

Status:
Enrollment to the global profiling study is closed and all active participants have completed their participation in the study. Enrollment in the sub-study was closed as of July 27, 2007. Data analysis is ongoing.

Manuscript in preparation:
- Voeghtly LM, Neatrour DM, Decewicz DD, Burke A, Haberkorn MJ, Patney HL, **Vernalis MN**, Ellsworth DL. Improvement in cardiometabolic risk factors during an intensive cardiovascular lifestyle intervention. (in preparation)

Subject Enrollment and Demographics
Subject enrollment was 374. There were 166 participants taking part in the lifestyle change program, 140 subjects serving as the control group, and 68 participants enrolled in the Sub-study. Demographic characteristics of the control group were: average age of 63.7 years, 51% were female, 29% were veterans or the spouse of a veteran, and 34% had diagnosed coronary heart disease.

Data:
Inflammation biomarker panel – Inclusion of dietary data in the manuscript in preparation for Nutrition, Metabolism, and Cardiovascular Diseases delayed the manuscript submission. The dietary data collection software was updated to the newest version available and required a laborious process of manual re-entry of all food diaries. All of this data has now been re-entered and re-analyzed.

Because so much effort was needed to re-enter the Ornish dietary data for the Insulin/Leptin biomarker study, it was decided to extract all dietary data from the food diaries, which will allow us to evaluate the major nutrient components of their diets (calories, carbohydrates, protein, and fat), as well as additional macronutrients, minerals, and vitamins. This data has been collected and preliminary analysis shows that there are significant differences in nutrients between Intervention and Control diets, which may contribute to cardiovascular disease reversal.

This study will evaluate if dietary levels of these nutrients are significant for the prevention or reversal of cardiovascular disease risk. For this purpose, we evaluated the dietary information

for several macronutrients, vitamins, and minerals from three-day food diaries at each of three distinct time points (Baseline, Week 12, and Week 52). We collected data on 152 participants (control n=76, intervention n=76) at 456 total time points; however, 22 time points were excluded due to missing food diary entries.

Nutrient variables (n=37) were measured using the ESHA Food Processor software version 8.4.0 (Table 8 below). Twenty-eight of these variables were found to be significantly different between Ornish participants and controls. After a review of the literature and meetings with a registered dietician, we selected six variables of specific interest for this study: cholesterol, vitamin B6, potassium, folate, manganese, and thiamin.

Cholesterol – Our results show that an Ornish diet decreases dietary cholesterol compared to control diets. Hypercholesterolemia is a known risk factor for cardiovascular disease, and there is a dramatic decrease in dietary cholesterol when participants follow a strict vegetarian diet compared to controls that eat their normal diet.

Vitamin B6 and Folate – Low levels of B vitamins can lead to homocysteine accumulation and increased blood coagulation, which is why increased homocysteine levels have been characterized as an independent risk factor for cardiovascular disease. Dietary vitamin B supplementation is recommended to reduce cardiovascular disease risk. Vitamin B6 and folate levels are significantly higher in Ornish participants compared to controls.

Potassium – Potassium is an electrolyte required for normal electrical activity in the heart and for maintaining normal blood pressure. Increasing dietary potassium, as we observed in the Ornish Program, can reduce blood pressure and therefore may reduce cardiovascular risk.

Manganese – Manganese is a trace element that plays a role in lipid and carbohydrate metabolism, cholesterol regulation, and ultimately atherosclerosis. Manganese levels were significantly increased in the diet of participants compared to controls.

Thiamin – Thiamin is related to diabetes, an independent risk factor for cardiovascular disease. Those with type I and type II diabetes have been shown to have ~75% lower serum thiamin concentrations than non-diabetics. Thiamin deficiency increases microvascular complications by making vascular cells more susceptible to the adverse effects of hyperglycemia. Our study shows that Ornish participants had a significantly higher amount of thiamin in their diets, which could indirectly lead to a reduction in cardiovascular disease risk.

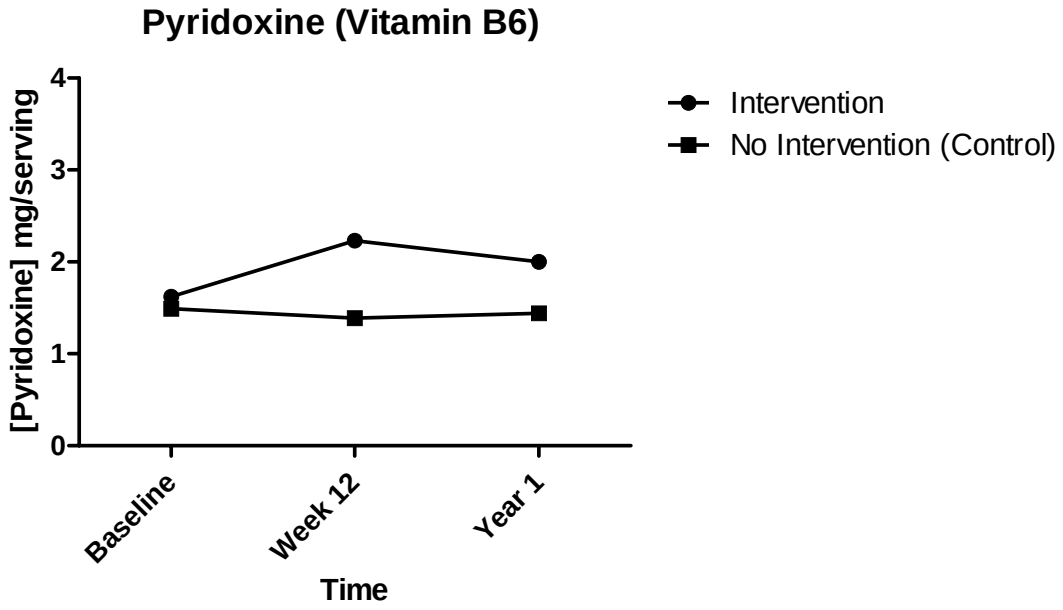
Based on our preliminary results, an Ornish diet is comprised of a beneficial balance of macronutrients, vitamins, and minerals that may be associated with a reduction in cardiovascular disease risk.

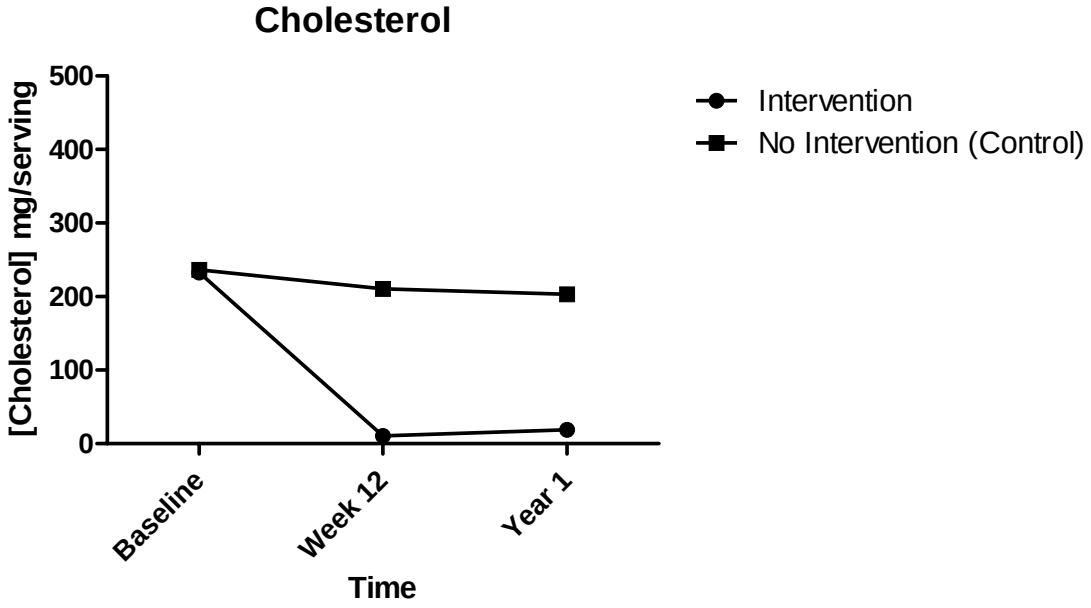
Table 8. Dietary intake of macronutrients, vitamins, and minerals in participants versus controls

Macronutrient	P-value
Kilocalories	0.3854
Protein	0.1164
Carbohydrate	< 0.0001
Fat, Total	< 0.0001
Alcohol	0.0011
Cholesterol	< 0.0001
Saturated Fat	< 0.0001
Monounsaturated Fat	< 0.0001
Polyunsaturated Fat	0.0503
Dietary Fiber, total	< 0.0001
Sugar, Total	< 0.0001
Other Fats	0.046

Vitamins	P Value
Vitamin A (RE)	< 0.0001
Vitamin C	< 0.0001
Vitamin D (µg)	0.0016
Vitamin E (ATE)	0.007
Thiamin	< 0.0001
Riboflavin	< 0.0001
Niacin	0.2409
Pyridoxine (Vitamin B6)	< 0.0001
Folate	< 0.0001
Cobalamin (Vitamin B12)	0.7891
Biotin	0.4181
Pantothenic Acid	0.0017
Vitamin K	0.0004

Minerals	P Value
Sodium	0.8394
Potassium	< 0.0001
Calcium	< 0.0001
Iron	< 0.0001
Phosphorus	< 0.0001
Magnesium	< 0.0001
Zinc	0.0001
Copper	< 0.0001
Manganese	< 0.0001
Selenium	0.3515
Chromium	0.4381
Molybdenum	0.0002


Pyridoxine (Vitamin B6)
[Pyridoxine] mg/serving
4
3
2
1
0
Baseline
Week 12
Year 1
Time
Intervention
No Intervention (Control)


Cholesterol
[Cholesterol] mg/serving
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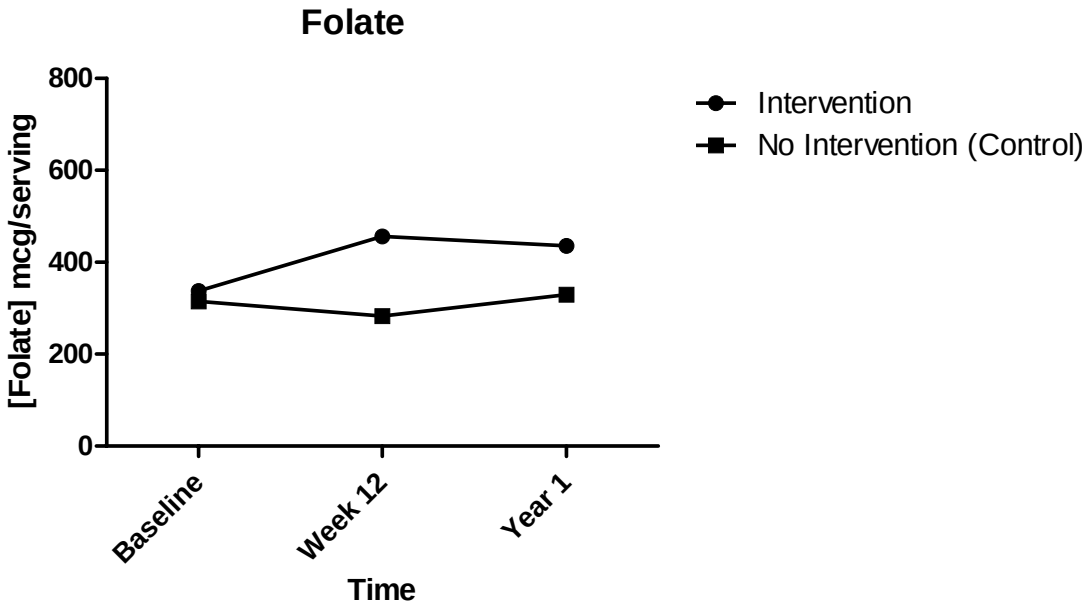
400
300
200
100
0
Baseline
Week 12
Year 1
Time
Intervention
No Intervention (Control)


Folate
[Folate] mcg/serving
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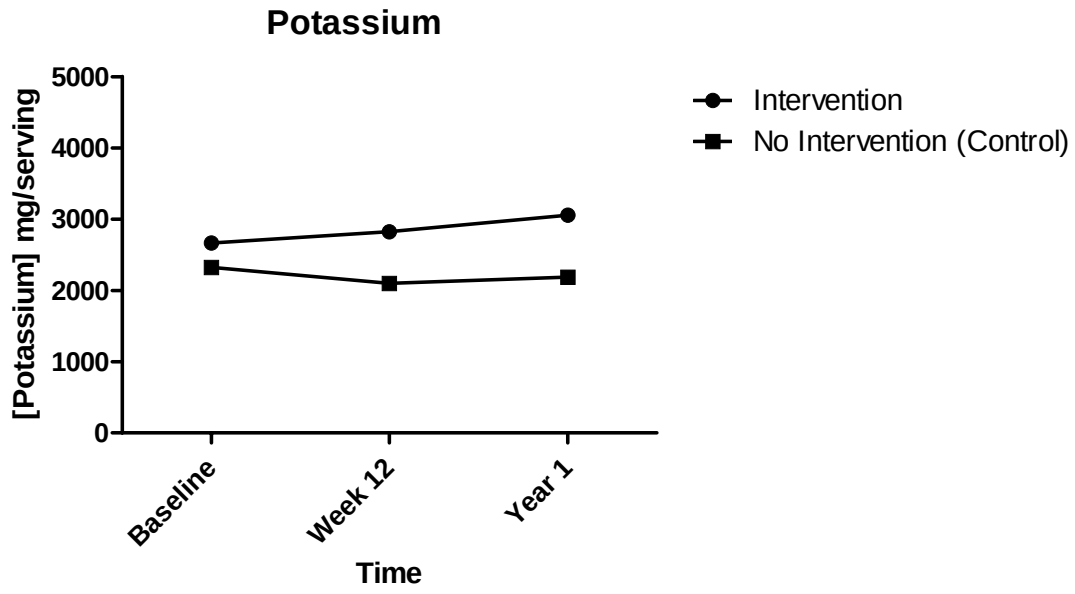
600
400
200
0
Baseline
Week 12
Year 1
Time
Intervention
No Intervention (Control)


Potassium
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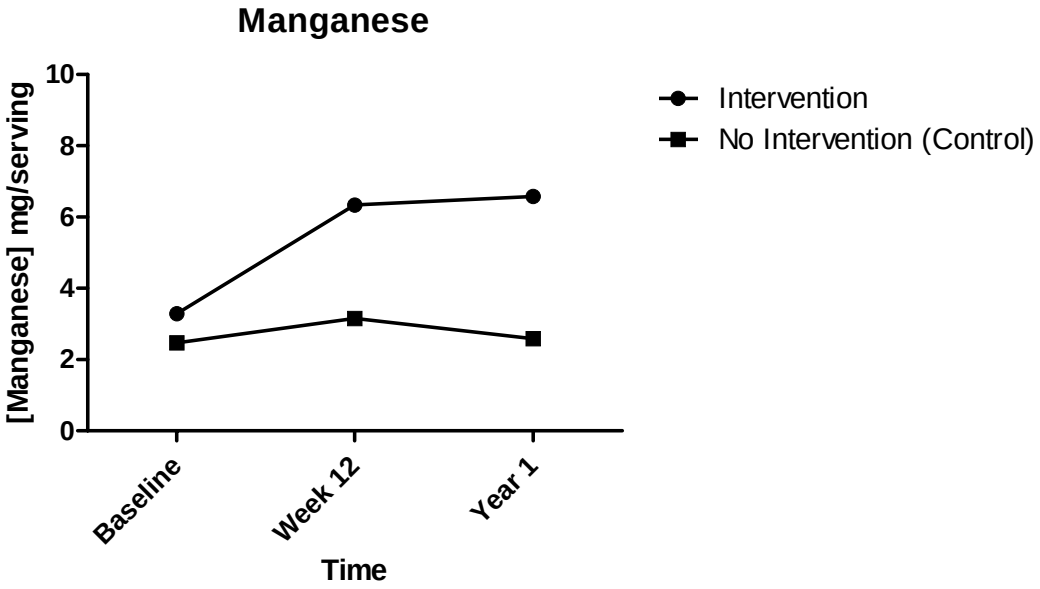
4000
3000
2000
1000
0
Baseline
Week 12
Year 1
Time
Intervention
No Intervention (Control)


Manganese
[Manganese] mg/serving
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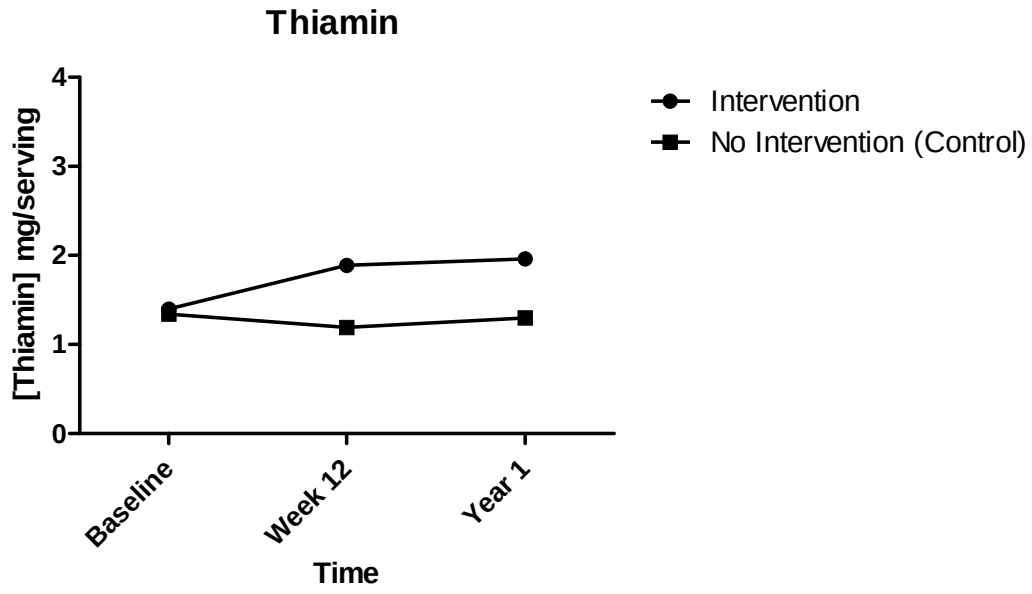
8
6
4
2
0
Baseline
Week 12
Year 1
Time
Intervention
No Intervention (Control)


Thiamin
[Thiamin] mg/serving
4
3
2
1
0
Baseline
Week 12
Year 1
Time
Intervention
No Intervention (Control)

Macrophage migration inhibitory factor (MIF) – MIF is an inflammatory cytokine that regulates smooth muscle cell migration and proliferation, and thus plays an important role in promoting development of atherosclerotic lesions. MIF has been shown to be an important biomarker for diseases with inflammation, such as CVD, diabetes, obesity, and cancer. Previous results showed that MIF levels decreased significantly ($p<0.05$) in Ornish participants compared to controls at 12 weeks, but there was no difference in MIF levels between cases and controls at one year. Stratification by gender indicated that only women who participated in the Ornish program showed significant reductions in MIF levels at 12 weeks (-23%). Men who participated in the program showed minimal change in MIF at 12 weeks (-2.3%).

Transcription of the human MIF gene is regulated by genetic polymorphisms in the MIF promoter, including the –173G/C single-nucleotide polymorphism and a sequence of tetra-nucleotide repeats at –794 (-794CATT$_{5-8}$). These polymorphisms may have relevance to cardiovascular disease, and this area has become a growing area of investigation.

A number of investigators examining the human MIF promoter genotypes have shown some evidence that MIF genetic variation is associated with coronary artery disease, although the degree of influence may well vary according to specific MIF alleles, populations, ethnicities, and other co-morbidities, such as diabetes mellitus or familial hypercholesterolemia.

During this period, we conducted further analysis on the MIF data, which incorporated genetic information on the –173G/C SNP and –794CATT repeat. First, we evaluated baseline MIF values for the CATT$_7$ allele versus all other CATT alleles and for the CC-GC versus GG SNP genotypes. Because this data was not normally distributed and could not be log-transformed into a normal distribution, we used a non-parametric Mann-Whitney U test for these analyses. No significant differences in the baseline data were observed for either polymorphism. We then used repeated-measures ANOVA by cohort type (Ornish or control) and CATT polymorphism within cohort type (Table 9). These analyses indicated a significant ($p<0.05$) change in MIF levels from Baseline to Week 12 in Ornish participants only, not in controls. Interestingly, MIF levels decreased 29% in participants who carried the –794CATT7 allele, but decreased only ~5% in participants who did not carry at least one CATT 7 allele.

Table 9. Change in plasma MIF levels by cohort type and –794CATT genotype

Cohort Type	CATT7 Value	n	Baseline[e]	Week 12[e]	% Change B to 12 Wk	Year 1[e]	% Change B to 1 Yr.	Between Factor Value[a]	
								B to W12 P Value	B to Y1 P Value
Control	No	63	2.8±1.9	3.0±2.3	8.3%	2.8±1.8	-0.1%	0.501	0.600
	Yes	20	3.2±1.8	3.2±2.0	-0.4%	3.4±2.4	5.9%		
Ornish	No	63	2.8±1.7	2.7±1.9	-5.2%	2.9±2.0	4.3%	0.026	0.993
	Yes	18	3.5±2.4	2.5±1.4	-29.3%	3.6±2.4	2.6%		

Gene Expression – During this period, we analyzed the second set of gene expression data from 89 Ornish participants (matched and unmatched) who had data at all three time points.

As before, integrity of the microarray gene expression data was assessed by rigorous QC. CEL files from all time points were imported into Partek® Genomics Suite v6.5 (Partek Incorporated, St. Louis, MO). Probe set intensities were obtained by Robust Multichip Algorithm (RMA) background correction, quantile normalization, median polish summarization, and $\log_2$ transformation. To assess data integrity, the processed intensity data was subjected to standard GeneChip® quality control parameters, which evaluated assay performance and ensured suitability for analysis. All arrays passed the quality control assessment and thus were included in further analyses.

Differential gene expression analysis between time points (baseline—12 weeks, baseline—52 weeks) was conducted using ANOVA with participant as the random effects factor and time point as the fixed effects factor. Resulting p-values were adjusted by FDR correction for multiple testing. Stringent gene lists were generated through combined significance (FDR-adjusted $p<0.05$) and expression change ($\geq$1.1-fold) filtering.

Functional enrichment analysis was performed on the stringent gene lists using Gene Ontology (GO) annotations to summarize the most enriched biological processes. The GO annotations were ranked by an enrichment p-value, which identified biological processes represented more frequently than expected by chance among genes that changed significantly in expression during the Ornish program.

Data analysis examined differential gene expression in three separate analyses: 1) diabetics, non-diabetics with high insulin, and non-diabetics with low insulin; 2) individuals diagnosed with clinical stress and unstressed participants; and 3) participants with the highest weight loss and participants with the least weight loss. Significant changes in expression were determined as a change in expression from Baseline to Week 12 or Baseline to Week 52 that was $\geq$1.1-fold at a False Discovery Rate (FDR) $p<0.05$

Below is a summary of results:

1. Diabetics (n=25), non-diabetics with high insulin (n=29), non-diabetics with low insulin (n=33)
    -- Diabetics
        0 genes at 12 weeks; 0 genes at 52 weeks
    -- Non-diabetics, high insulin
        30 genes at 12 weeks; 39 genes at 52 weeks
    -- Non-diabetics, low insulin
        0 genes at 12 weeks; 0 genes at 52 weeks

All of the changes in gene expression were restricted to the non-diabetics with high insulin. Functional enrichment analysis showed enrichment in defense response genes at 12 weeks and genes regulating symbiosis at 52 weeks.

For cross-group comparisons, we saw:

At 12 weeks

-- Diabetics vs high insulin:	0
-- Diabetics vs low insulin:	201
-- High insulin vs low insulin:	0

At 52 weeks

-- Diabetics vs high insulin:	0
-- Diabetics vs low insulin:	4
-- High insulin vs low insulin:	0

2. High stress (n=29; PSS $\geq$18), low stress (n=60, PSS <18)
    -- High stress

6 genes at 3 months; 0 genes at one year

-- Low stress

26 genes at 3 months; 29 genes at one year

Most of the changes in gene expression were observed in the low-stress group. Similar to the diabetics vs non-diabetics analysis, functional enrichment analysis showed enrichment in defense response genes and genes regulating symbiosis at 12 weeks and 52 weeks.

3. Successful weight loss (top two pentiles, n=35), unsuccessful weight loss (bottom two pentiles, n=36)

-- Successful weight loss

41 genes at 3 months; 3223 genes at one year

-- Unsuccessful weight loss

0 genes at 3 months; 0 genes at one year

Clearly, individuals who lost the most weight showed dramatic changes in gene expression. Many genes were involved in immune and defense response as well as endosymbiosis at 12 weeks. At 52 weeks, most genes affected carbohydrate and cholesterol metabolism. A heat map depicting the degree of molecular change is presented in Figure 2 below.

In the coming year, validation qRT-PCR experiments will be performed to confirm differential gene expression detected by microarray analysis. Total RNA (500 ng) will be reverse-transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA). Resulting cDNA (10 ng) will be subjected to qRT-PCR using TaqMan® Gene Expression Assays (Applied Biosystems) according to the manufacturer's protocol on an iCycler iQ™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA). All samples will be run in duplicate for each assay and the mean value of the duplicate assays was analyzed by the $\Delta\Delta C_T$ method, which determines levels of expression for each target gene at each time point.

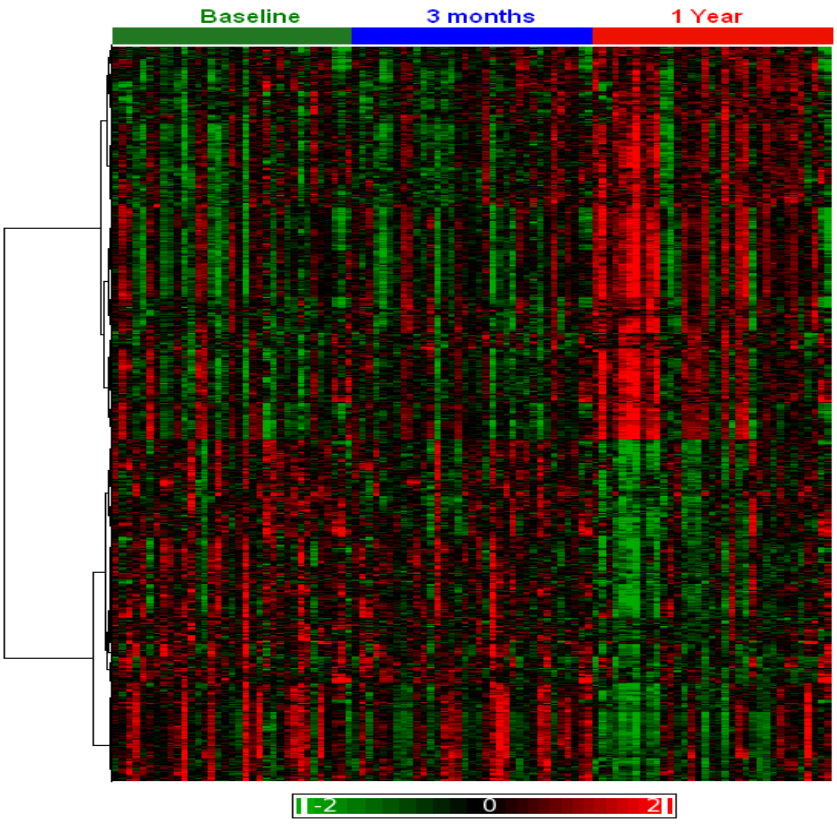


Figure 2. Heat map showing gene expression changes with weight loss

Plasma Metabolites – We are continuing our collaboration with Dr. Dean Jones and Dr. Quinlyn Soltow at Emory University to profile plasma metabolites associated with CVD development. To date, we analyzed a total of 17 Ornish patients and 17 matched controls (all at three time points) by liquid chromatography-Fourier transform mass spectrometry (LC-FTMS). All assays were run in duplicate. We plan to prepare a manuscript on these results in the coming year.

During this period, the following abstract was *presented* as a poster (See Appendix A):

- Ellsworth DL, Soltow QA, Kolli K, Patney HL, Jones DP, and Vernalis MN. Cardiac rehabilitation involving lifestyle modification alters comprehensive plasma metabolomic profiles defined by LC-FTMS. AHA Nutrition, Physical Activity & Metabolism/CV Disease Epidemiology and Prevention Scientific Session, Mar 2011: Atlanta, GA.

Structural and Functional Measures of Cardiovascular Health

Specific endpoints measured include ejection fraction and wall motion, coronary artery calcification scores, left and right ventricular volumes, myocardial mass, stenosis sizing and vessel diameter, plaque density and differentiation of calcified versus non-calcified plaque, and tissue perfusion and viability. Work continues on the quantification and interpretation of the huge volumes of imaging data we have acquired.

Proteomics

As the proteomics core facility at WRI was disbanded during the recent quarter, no updates on our proteomics research are available at this time.

Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal, Sub-Study for Previous Subjects in the Dr. Dean Ornish Program for Reversing Heart Disease.

The primary objective of this study is to examine associations between DNA variation (in the form of 500,000+ single nucleotide polymorphisms) and participant response to the program. We are examining the influence of innate genetic variation on overall response, quantified as the risk of future cardiac events (Framingham risk), as well as response of specific cardiovascular disease risk factors. The main hypothesis is that innate variation in genes associated with lipid metabolism, protein biosynthesis, protein modification, transcription regulation and/or cell surface receptors (or other genes) will correlate positively with response to intensive lifestyle changes involving diet, exercise, meditation, yoga and group support, which may lead to improved CHD risk factor profiles and genetic markers of coronary artery disease reversal or stabilization. Participants in this study are being recruited from previous cohorts of the Dr. Dean Ornish Program for Reversing Heart Disease at Windber Medical Center (prior to implementation of the primary Molecular Profiling Protocol described above).

Status:

During the past quarter, we profiled individual SNPs defined in recent genome-wide association studies to have an impact on plasma lipids other than triglycerides [high density lipoprotein (HDL-) cholesterol, low density lipoprotein (LDL-) cholesterol, and total cholesterol]. All of the SNPs were selected from previously published genome-wide association studies. We are determining if SNPs that have been shown to influence lipid traits in the general population influence how these traits respond during participation in the Ornish program.

A total of 2,778 DNA samples were genotyped during the quarter, with an additional 76 reruns. As all genotypes were assayed in duplicate, a total of 5,708 genotypes were generated. Genotypes were generated for 16 SNPs using the new Applied Biosystems ViiA 7™ Real-Time PCR System. The following SNPs were analyzed for the corresponding traits: rs11206510 – LDL cholesterol, rs12740374 – LDL cholesterol, rs599839 – LDL cholesterol, rs515135 – LDL cholesterol, rs12654264 – LDL cholesterol, rs1501908 – LDL cholesterol, rs12670798 – LDL cholesterol and total cholesterol, rs4149268 – HDL cholesterol, rs2338104 –

HDL cholesterol, rs1532085 – HDL cholesterol, rs1800588 – HDL cholesterol, rs3764261 – HDL cholesterol, rs255052 – HDL cholesterol, rs4939883 – HDL cholesterol, rs688 – LDL cholesterol, rs157580 – LDL cholesterol and total cholesterol. Statistical analysis of these SNPS will be conducted during the upcoming quarters.

In addition to the above SNPs, over the past period of performance we have profiled 27 SNPs defined in recent genome-wide association studies to have an impact on CVD development or associated risk factors such as weight, blood pressure, or lipids. We observed no relationship between SNPs influencing BMI and blood pressure and response to the Ornish program. Numerous SNPs influencing triglycerides showed a differential response to the program (manuscript in preparation). Below in Table 10 is a summary of the 19 SNPs from recent genome-wide association and confirmation studies with robust statistical evidence for association with plasma triglyceride levels in the general population and a minor allele frequency (MAF) $\geq$5% in populations of European descent in the National Center for Biotechnology Information (NCBI) Entrez SNP database.

Of the 19 SNPs examined, two SNPs (rs442177 and rs17145738) appeared to influence plasma triglycerides prior to enrollment in the program, as triglyceride levels for both SNPs were significantly different ($p<0.05$) between genotypes at baseline (Table 11). Sixteen SNPs showed evidence of an influence on triglyceride response throughout the program — triglyceride levels changed significantly from Baseline to Week 12 and/or Baseline to Week 52 in participants with one genotype but not in those carrying the alternate genotype. For 3 SNPs, change in triglyceride levels during the program was significantly different between genotype groups at Week 12 (rs442177 and rs17145738) or at Week 52 (rs3846662 and rs17145738).

We investigated potential interactive effects of gender and genotype on triglyceride response by two-factor repeated measures ANOVA. At 4 SNPs (rs10889353, rs442177, rs3846662, and rs16996148) response differed significantly by gender for one genotype, but not the other genotype (Table 12). In all cases, triglycerides showed a much greater decrease in men compared to women.

Table 10. SNPs associated with plasma triglyceride levels in recent GWAS

SNP[a]	Chromosome	Position[b]	Location	Gene(s)[c]	Alleles (MAF)[d]	Effect size (mg/dl)[e]	References
rs10889353	1p31.3	63118196	Intron 4	DOCK7/ANGPTL3-ATG4C	A/c (0.33)	–4.9[f]	967,968,975,979,982
rs12130333	1p31.3	63191777	Intergenic	DOCK7/ANGPTL3-ATG4C	C/t (0.16)	–4.9[f]	965,968,974,975
rs4846914	1q41-q42	230295691	Intron 1	URB2-GALNT2-PGBD2	A/g (0.43)	+2.8	965,968
rs673548	2p24-p23	21237544	Intron 23	APOB	G/a (0.25)	–6.0[g]	967,968,976
rs1260326	2p23	27730940	Leu446Pro	GCKR	C/t (0.44)	+10.3	966,968,974,975,976,978,979
rs780094	2p23	27741237	Intron 16	IFT172-FNDC4-GCKR	G/a (0.45)	+8.6	965,966,967,969,971,975,976
rs442177	4q21	88030261	Intron 12	SLC10A6-AFF1-KLHL8	A/c (0.38)	–2.3	968,980
rs3846662	5q13.3-q14	74651084	Intron 13	ANKRD31-HMGCR-COL4A3BP	T/c (0.49)	—	967
rs17145738	7q11.23	72982874	Intergenic	BCL7B-TBL2-MLXIPL	C/t (0.11)	–8.2	965,966,968,975,976,978,982
rs328	8p22	19819724	Ser474X	LPL	C/g (0.10)	–9.4[h]	965,969,970,974,976,978,983
rs17321515	8q24.13	126486409	Intergenic	KIAA0196-NSMCE2-TRIB1	A/g (0.47)	–6.4	965,966,975,978,982
rs2954029	8q24.13	126490972	Intergenic	KIAA0196-NSMCE2-TRIB1	A/t (0.46)	–6.4	966,968,975,979,980
rs3905000	9q31.1	107657070	Intron 2	NIPSNAP3A/B-ABCA1-SLC44A1	G/a (0.13)	—	967
rs174547	11q12.2-q13.1	61570783	Intron 9	FEN1-FADS1-FADS2-FADS3	T/c (0.34)	–16.4[i]	975,982
rs12272004	11q23.3	116603724	Intergenic	BUD13-ZNF259-APO(A5/A4/C3/A1)	C/a (0.07)	+18.1[j]	967
rs964184	11q23.3	116648917	Intergenic	BUD13-ZNF259-APO(A5/A4/C3/A1)	C/g (0.13)	+18.1	966,968,975,979
rs10401969	19p13.11	19407718	Intron 8	TM6SF2-SUGP1(SF4)-MAU2	T/c (0.06)	–12.3	966,968
rs16996148	19p13.11	19658472	Intergenic	YJEFN3-CILP2-PBX4	G/t (0.05)	–6.1	965,966,975
rs439401	19q13.2	45414451	Intergenic	TOMM40-APOE-APOC1	C/t (0.36)	–5.5	967,968

[a] Underlined SNPs also have been associated (P<0.001) with increased risk of coronary artery disease (#966, 968).

[b] National Center for Biotechnology Information human genome reference assembly, Build 37.1.

[c] Nearest annotated genes and nearby biological candidate genes. For intergenic and intronic SNPs, the closest gene is underlined.

[d] Major allele, minor allele, and minor allele frequency (MAF) in this study. Alleles are designated with respect to the "+" strand.

[e] For SNPs rs1260326, rs780094, rs17145738, rs17321515, rs2954029, rs964184, rs10401969, and rs16996148 effect sizes were measured as additive effects corresponding to the average change in triglyceride levels when one major allele was replaced with one minor allele (#966). For SNPs rs4846914, rs442177, and rs439401 effect sizes were estimated as percent changes in triglyceride levels, from a mean triglyceride level of 137.9 mg/dl, due to a single copy of the minor allele (#968).

[f] Effect size, estimated as a percent change in triglyceride levels, from nearby SNP rs2131925 not examined in this study.

[g] Effect size, estimated as a percent change in triglyceride levels, from nearby SNP rs1042034 not examined in this study.

[h] See #983.

[i] See #982.

[j] Effect size, corresponding to actual change in triglyceride levels, from nearby SNP rs964184 examined in this study.

Table 11. Triglyceride levels at Baseline, Week 12, and Week 52 by SNP genotype.

SNP[a]	Genotype	n	Baseline (SD)	Week 12 (SD)	% Change	Between genotype p-value[b]	Week 52 (SD)	% Change	Between genotype p-value[c]
rs10889353 [1]	AA	77	190.3 (96.5)	168.4 (75.4)[d]	–11.5[d]	0.381	161.7 (72.7)[d]	–15.0[d]	0.093
	CC-CA	93	170.9 (90.2)	159.1 (76.6)	–6.9		162.4 (94.9)	–5.0	
rs12130333 [1]	TT-TC	51	159.3 (60.7)	155.7 (67.6)	–2.3	0.142	148.2 (59.0)	–7.0	0.475
	CC	119	188.4 (103.2)	166.6 (79.4)[d]	–11.6[d]		168.0 (94.0)[d]	–10.8[d]	
rs4846914 [1]	AA	65	183.6 (90.5)	160.7 (79.8)[d]	–12.5[d]	0.308	158.4 (73.9)[d]	–13.7[d]	0.229
	GG-GA	112	173.6 (93.4)	162.3 (73.5)	–6.5		162.7 (90.8)	–6.3	
rs673548 [2]	AA-AG	72	172.2 (84.8)	159.6 (66.0)	–7.3	0.570	155.2 (84.8)	–9.9	0.928
	GG	98	185.2 (99.2)	166.0 (82.8)[d]	–10.3[d]		167.1 (85.7)	–9.8	
rs1260326 [2]	TT	37	189.6 (95.0)	157.0 (59.3)[d]	–17.2[d]	0.108	158.1 (76.2)[d]	–16.6[d]	0.186
	CC-CT	136	175.9 (91.7)	165.2 (79.4)	–6.1		163.3 (87.4)	–7.2	
rs780094 [2]	CC	60	170.0 (90.8)	156.6 (73.6)	–7.9	0.774	143.5 (71.2)[d]	–15.6[d]	0.223
	TT-TC	118	181.7 (93.0)	164.9 (76.7)[d]	–9.3[d]		170.0 (89.6)	–6.4	
rs442177 [4]	CC	23	141.9 (58.3)[e]	160.7 (85.1)	+13.2	0.014	143.7 (74.6)	+1.2	0.198
	AA-AC	147	185.6 (96.5)[e]	163.7 (74.8)[d]	–11.8[d]		165.0 (86.7)[d]	–11.1[d]	
rs3846662 [5]	AA	39	170.0 (80.6)	154.6 (78.8)	–9.0	0.978	174.5 (109.5)	+2.7	0.049
	GG-GA	137	179.6 (95.9)	164.6 (75.3)	–8.4		156.8 (76.6)[d]	–12.7[d]	
rs17145738 [7]	TT-TC	38	208.2 (110.7)[e]	171.2 (70.1)[d]	–17.8[d]	0.039	165.2 (84.6)[d]	–20.7[d]	0.018
	CC	139	169.5 (85.4)[e]	160.0 (77.3)	–5.6		159.5 (85.0)	–5.9	
rs328 [8]	GG-GC	31	166.3 (77.9)	153.7 (63.4)	–7.5	0.747	156.5 (65.0)	–5.9	0.533
	CC	139	182.7 (96.4)	165.4 (78.6)[d]	–9.5[d]		163.3 (89.4)[d]	–10.6[d]	
rs17321515 [8]	GG	41	170.3 (73.0)	149.2 (66.0)	–12.4	0.589	143.5 (69.6)	–15.7	0.338
	AA-AG	137	180.0 (97.3)	166.0 (78.1)	–7.8		166.3 (88.1)	–7.6	
rs2954029 [8]	TT	37	166.7 (67.0)	152.7 (68.5)	–8.4	0.833	145.9 (72.3)	–12.5	0.699
	AA-AT	133	182.6 (99.0)	166.2 (77.9)[d]	–9.0[d]		167.3 (88.6)	–8.3	
rs3905000 [9]	AA-AG	45	180.7 (99.6)	168.8 (77.6)	–6.6	0.690	154.8 (68.9)	–14.3	0.353
	GG	133	176.8 (89.9)	159.8 (75.1)[d]	–9.6[d]		163.2 (89.4)	–7.7	
rs174547 [11]	TT	71	184.2 (92.7)	167.6 (74.7)	–9.0	0.982	166.7 (93.4)	–9.5	0.984

	CC-CT	99	176.5 (94.1)	160.2 (77.2)	–9.2		158.8 (79.3)	–10.1	
rs12272004 [11]	AC	25	172.2 (88.3)	170.8 (70.2)	–0.8	0.298	172.8 (122.0)	+0.4	0.392
	CC	152	178.7 (93.4)	161.0 (76.7)[d]	–9.9[d]		158.7 (77.2)[d]	–11.2[d]	
rs964184 [11]	GG-GC	45	175.7 (98.0)	166.6 (79.0)	–5.2	0.480	165.7 (99.8)	–5.7	0.577
	CC	128	178.5 (91.6)	160.3 (75.5)[d]	–10.1[d]		159.1 (80.4)[d]	–10.8[d]	
rs10401969 [19]	CC-CT	18	158.4 (69.1)	159.6 (59.5)	+0.8	0.302	167.6 (66.5)	+5.8	0.137
	TT	152	181.6 (95.4)	163.7 (77.8)[d]	–9.8[d]		162.1 (87.7)[d]	–10.7[d]	
rs16996148 [19]	TT-TG	16	161.0 (72.2)	173.6 (66.3)	+7.8	0.098	168.6 (68.4)	+4.7	0.173
	GG	154	181.7 (95.2)	162.2 (77.1)[d]	–10.7[d]		161.4 (87.0)[d]	–11.1[d]	
rs439401 [19]	CC	74	193.0 (115.0)	168.3 (82.6)[d]	–12.8[d]	0.200	181.1 (104.7)	–6.1	0.395
	TT-TC	96	169.5 (71.3)	159.5 (70.7)	–5.9		147.4 (63.4)[d]	–13.0[d]	

[a] Chromosome location in brackets.

[b] From independent samples t-test (two-tailed) comparing genotypes at Baseline and Week 12.

[c] From independent samples t-test (two-tailed) comparing genotypes at Baseline and Week 52.

[d] Significantly different from Baseline at $p<0.05$.

[e] Baseline values significantly different ($p<0.05$) between genotypes.

Table 12. Triglyceride levels at Baseline, Week 12, and Week 52 for selected SNPs by genotype and gender.

SNP[a]	Genotype	Gender	n	Baseline (SD)	Week 12 (SD)	% Change	Between gender p-value[b]	Week 52 (SD)	% Change	Between gender p-value[c]
rs10889353 [1]	AA	F	34	197.7 (105.6)	179.1 (91.8)	–9.4	0.742	173.9 (85.7)	–12.1	0.611
		M	43	184.4 (89.4)	160.0 (59.2)	–13.3		152.1 (59.9)[d]	–17.6[d]	
	CC-CA	F	48	159.4 (73.2)	165.2 (72.9)	+3.7	0.013	168.0 (106.2)	+5.4	0.029
		M	45	183.2 (104.9)	152.5 (80.7)[d]	–16.8[d]		156.4 (81.8)	–14.7	
rs442177 [4]	CC	F	14	142.1 (48.4)	171.8 (81.0)	+20.9	0.328	143.9 (63.4)	+1.3	0.991
		M	9	141.7 (74.4)	143.3 (93.4)	+1.2		143.2 (93.7)	+1.1	
	AA-AC	F	68	182.1 (94.6)	170.8 (81.6)	–6.2	0.106	175.9 (102.9)	–3.4	0.040
		M	79	188.6 (98.6)	157.6 (68.3)[d]	–16.4[d]		155.5 (69.3)[d]	–17.5[d]	
rs3846662 [5]	AA	F	23	176.9 (86.2)	159.8 (83.9)	–9.6	0.849	181.7 (127.8)	+2.7	0.979
		M	16	160.1 (73.3)	147.1 (72.9)	–8.1		164.2 (78.8)	+2.5	
	GG-GA	F	62	173.7 (89.7)	175.8 (78.7)	+1.2	0.016	165.8 (83.3)	–4.6	0.029
		M	75	184.4 (101.2)	155.3 (71.5)[d]	–15.8[d]		149.4 (70.2)[e]	–19.0[e]	
rs17145738 [7]	CC	F	71	170.2 (88.5)	168.0 (80.8)	–1.3	0.212	169.8 (98.1)	–0.3	0.138
		M	68	168.7 (82.6)	151.6 (73.0)	–10.1		148.8 (67.7)	–11.8	
	TT-TC	F	14	196.7 (86.5)	188.9 (75.8)	–4.0	0.083	171.7 (93.1)	–12.7	0.223
		M	24	214.9 (124.0)	160.8 (66.0)[d]	–25.2[d]		161.4 (81.0)[d]	–24.9[d]	
rs16996148 [19]	GG	F	77	178.0 (91.7)	173.1 (82.9)	–2.8	0.012	172.0 (99.5)	–3.3	0.022
		M	77	185.3 (99.1)	151.4 (69.6)[e]	–18.3[e]		150.8 (71.5)[e]	–18.6[e]	
	TT-TG	F	5	134.0 (8.6)	138.8 (32.6)	+3.6	0.811	146.6 (65.5)	+9.4	0.861
		M	11	173.3 (85.3)	189.5 (72.7)	+9.3		178.6 (70.3)	+3.0	

[a] Chromosome location in brackets.

[b] From independent samples t-test (two-tailed) comparing gender within genotypes at Baseline and Week 12.

[c] From independent samples t-test (two-tailed) comparing gender within genotypes at Baseline and Week 52.

[d] Significantly different from Baseline at $p<0.05$.

[e] Significantly different from Baseline at $p<0.001$.

Task #10: Continuation of the "Comprehensive Cardiovascular Risk Assessment and Prevention Program (CPP)".

This program serves as a platform for ongoing translational research activities, a "virtual laboratory" based on scientific findings for the development of best personalized preventive practices. In other words, the platform allows ICHP to gather an expansive number of data points for each patient or subgroup of patients (eventually combined with data at a molecular level) that when leveraged will result in the creation of new tools in technology to define wellness, predict and prevent disease, and empower patients and providers to transform their healthcare.

The CPP platform has a dual purpose and is multifunctional. This platform 1) allows for multiple research protocols to be conducted as it sets the stage for recruitment, enrollment and hypothesis generation, advanced data modeling and simultaneously 2) provides a venue where research findings from these protocols can then be tested, validated and translated into application for clinical practice. Our protocols within the CPP are specifically designed to examine the effects of our military's high op tempo which predisposes our service members to accelerated atherosclerotic risk resulting from high stress, PTSD, depression, sleep insufficiency, overweight, prediabetes and prehypertension among other traditional disease risk factors.

This program was established to address the unique needs of military beneficiaries at risk for CV disease. It includes conventional and novel CV risk profiling (health assessments, labs, markers, wearable monitors) and tailored, personalized behavioral recommendations for primary or secondary prevention by an integrative team of providers comprised of a cardiologist, sleep specialist, nurse practitioners, nutritionists, stress management instructors and exercise physiologists. Validated tools to screen for and measure CV risk are part of this inclusive package. Report cards for the patient and provider as well as email notifications are utilized. The program is an adjunct to the best medical practices provided by their primary care provider. Up to 1000 patients may be enrolled each year. Some of the patients (such as nurses or medical holdovers etc) may be in subgroup programs because of unique needs. The CPP serves as a platform for ongoing translational research activities, a "virtual laboratory" for the development of best preventive practices and for CV educational and marketing materials.

Status:

Total patient visits: 1,458 + 757 telephonic visits = 2,215 in past year; (253 + 125 telephonic contacts= 378 during Jun 11- Aug 11).

From August 2010-August 2011, customer satisfaction surveys continued to average a score of 3.95 out of 4.0, demonstrating high patient satisfaction even in the face of the impending move from WRAMC Washington DC campus to the WRNMMC Bethesda campus.

The following abstracts were *presented* as posters at national meetings in the past year (See Appendix A):

- Eliasson A, Kashani M, Hoffman J, Vernalis M. Racial differences in perceived stress, sleep habits, and daytime symptoms. Associated Professional Sleep Societies, Jun 2011: Minneapolis, MN.
- Kashani M, Eliasson A, Vernalis M. Sleep parameters associated with hyperinsulinemia increase cardiovascular disease risk. National Sleep Foundation, Mar 2011: Washington, DC.
- Eliasson A, Kashani M, Bailey K, Vernalis M. The Berlin questionnaire identifies a population with traits inhibiting adherence. American Thoracic Society, May 2011: Denver, CO.
- Mayhew M, Eliasson A, Kashani M, Vernalis M. Should Subclinical Hypothyroidism Be Treated to Lower Cardiovascular Risk? American College of Nurse Practitioners, Oct 2010.

- Modlin R, Kashani M, Eliasson A, Bailey K, Vernalis M. The need for cardiovascular prevention in young service members. Army American College of Physicians, Nov 2010: Bethesda, MD.
- Vernalis M, Kashani M, Eliasson A. The need for cardiovascular prevention in young service members. Force Heath Protection Conference, August 2010: Phoenix, AZ.

The following abstracts were *presented* as podium presentations at national meetings in the past year (See Appendix A):

- Eliasson A, Kashani M, Mayhew M, Ude A, Hoffman J, Vernalis M. Reducing Perceived Stress Improves Sleep Quality—A Longitudinal Outcomes Study. CHEST. Oct 2010.
- Modlin, R, Kashani, M, Eliasson A, Vernalis, M. Comprehensive, Early Assessment is Critical for Cardiovascular Prevention. Army ACP, 2010.

Manuscript accepted for publication:

- Kashani M, Eliasson A, Vernalis M. Perceived stress correlates with disturbed sleep—a link connecting stress and cardiovascular disease. *Stress: the International Journal on the Biology of Stress*. 2011;19 June [epub ahead of print].

1st quarter:

- Analysis of Active Duty (AD) subgroups: Total Soldier Concept
  - Refined process for workshop, appointments, data collection, coaching, follow-up and aggregate report to Senior Enlisted Advisor
  - Refined tracking system for admin staff to follow flow of pts
- Designed new "Empowerment Train" schematic used to guide program flow
- Continued CPP Optimization Initiative (clinical & administrative)
  - Designed new Interactive Educational Workshop
  - Re-established customer service training for Admin staff
  - Designed motivational incentive sequence for patients
  - Tracked productivity of clinical and admin staff with new system
  - Performed 5% chart reviews quarterly- provided recommendations
  - Refined Clinical Review process and data capture of metrics
- Continued clinical enhancement of adding CIMT on all pts
- Continued ICHP Database and Platform Creation: Designed and communicated infrastructure of database to support the conceptual, logical, dimensional aspects of our clinical mission
- Our alumni continue to validate our successful model of care while they return for visits to the CPP/ ICHP to take part in our Healthy Cooking Demonstrations using our *Healthy Cooking Guide* designed to provide AD with practical tips for healthy living.

2nd quarter:

- Data management of subgroups for preclinical states (prehypertension, prediabetes, subclinical hypothyroidism)
- Creation of new track- Prevention Empowerment/Education Plan for Prediabetic and Diabetic patients as an adjunct to already established four dimensional approach to prevention.
- Re-enforced "Empowerment Train" schematic to guide appointment and behavioral compliance
- February 2011: Heart Health Month: nurses provided blood pressure screening and education for Active Duty and other beneficiaries based on recent AHA blood pressure reduction initiatives
- Continued CPP Optimization Initiative (clinical & administrative)
  - Implemented new Interactive Educational Workshop
  - Re-enforced customer service training for Admin staff
  - Implemented motivational incentive sequence for patients
  - Tracked productivity of clinical and admin staff with new system
  - Performed 5% chart reviews quarterly- provided recommendations

- o Refined Clinical Review process and data capture of metrics

3rd quarter:

- Revision of clinical guidelines to update CV health screening practices.
- Continued development of new track to improve glucose regulation- Prevention Empowerment/Education Plan for Prediabetic and Diabetic patients as an adjunct to already established prevention practices.
- Created plan for implementation of clinical services and quality assurance of prevention model at WRNMMC.
- Investigated new approaches to behavior modification sustainment through multimedia approaches to learning.
- Continued ICHP Database and Platform Creation: discussed metrics and flow with HJF regarding design and infrastructure of database to support the conceptual, logical, dimensional aspects of our clinical mission. Identified new IT needs to support current initiatives and move to WRNMMC.

4th quarter:

- Completed writing of DCI formatted protocol including consent form for protocol entitled ZENITH in collaboration with Co-PI in Cardiology Service.
- ICHP research Database: Functional requirements discussed with HJF. Awaiting vendor selection. Data variables outlined and streamlined for database creation. Personnel responsible for management of database identified, new job description written.
- Investigating possible statistical support for analysis of data, maintenance of ICHP publication plan, and generation of more detailed patient outcome clinical reports.
- Creation of new ICHP Website in order to support transition to the new WRNMMC location and to maintain communication with research participants.
- Significant innovations in Clinical Flow Plan of CPP, refinement of processes.
- Creation of new clinical positions to support expanding research initiatives: Integrative Health Coach, Data Outcomes Specialist, Clinical Outcomes Registered Nurse.
- Performed 5% chart reviews- Identified need for specific track for pre-diabetes and diabetes care.
- Development of pre-diabetes and diabetes clinical track using novel team approach of integrative care.
- Clinical Transition Strategy Plan for smooth transition and to re-establish high standards of our DOD COE at WRNMMC to serve all military beneficiaries.

Recent analysis of relevant clinical data shows:

1. In assessing multiple CV risk factors and health behaviors in a group of young soldiers with hypertension utilizing laboratory studies, anthropometrics, validated questionnaires and sophisticated actigraphic devices to measure activity levels and sleep. Of 12 participants (7 men, average age 27.8 years, 8 Caucasian, 3 African-American, 1 other), 7 had systolic hypertension or diastolic hypertension (6 had both). These hypertensive soldiers were older (32 vs 22 years, p=0.02) and had multiple risk factors for metabolic syndrome: Dyslipidemia (total cholesterol 198 vs 153 mg/dL, p=0.02; LDL cholesterol 122 vs 83 mg/dL, p=0.03); Obesity (BMI 31.9 vs 27.8 kg/m^2, p=0.19, WC 105 vs 85 cm, p=0.01, and % body fat 37 vs 29%, p=0.26); and Glucose Dysregulation (insulin 26 vs 9 uIU/ml, p=0.26; HOMA 5.8 vs 1.7, p=0.27). The hypertensive soldiers also had higher prevalence of sleep apnea (43 vs 0%, p=0.11), and were at markedly higher risk for CV disease (ICHP CV Risk Score 10.3=moderate risk vs 4.8 points=low risk, p=0.02). There were no significant differences in sleep time but soldiers with normal blood pressure exercised ½ hour more each day compared to hypertensive soldiers. **The findings demonstrate clustering of multiple risk factors for CV disease in young soldiers emphasizing the need for comprehensive early assessment for CV disease prevention. In view of the multiple co-morbid risk factors, the use of an integrative intervention strategy may be highly effective.**

2. In examining CV risk in a group of young AD members, of 14 participants (9 men), average age 27.7 years, 5 had abnormal CIMT. These five soldiers exercised less (524±183 MET-min/week versus 1577±1253, p=0.10), showed more snoring/OSA (60% versus 11%, p=0.05), weighed more (BMI=32.4±5.6 kg/m^2 versus 28.8±4.0, p=0.18), had dyslipidemia (100% versus 33%, p=0.01), lower HDL (43.2±11.8 mg/dL versus 55.7±11.4, p=0.08), and lower vitamin D (12.5±4.7 pg/mL versus 20.0±7.9, p=0.08). **In this cohort of young soldiers, subclinical atherosclerosis was prevalent. Reversible risk factors were identified with easily obtained and inexpensive assessment tools. Our experience supports earlier assessment and prevention to conserve the Fighting Force.**

3. We sought to evaluate the important health associations of Subclinical hypothyroidism in subjects entering our CPP. Of 340 consecutive patients, 51 (15%) were excluded for diagnosed thyroid disease or thyroid replacement medication. The remaining 289 patients (165 women) comprised the study set with 111 Caucasian, 89 African-American, 12 Hispanic, 2 Asian and 75 undeclared. There were 10 patients (3.5%) with SCH (6 women, mean TSH 4.74±0.41) and 279 patients with normal thyroid studies (158 women, mean TSH 1.78±0.82). For patients with and without SCH, two sample t-tests showed no differences in BMI, waist circumference, perceived stress levels, or C-reactive protein. Indices of glucose metabolism between groups were not statistically different, including fasting glucose, HbA1c, and HOMA. Compared to normal subjects, patients with SCH showed no differences in sleep habits and symptoms, including sleep latency, sleep duration, habitual snoring, risk for sleep apnea, daytime sleepiness and fatigue. Lipid studies showed no statistical differences in total cholesterol (p=0.55), LDL (p=0.71), HDL (p=0.16), TG (p=0.77), PLA2 (p=0.18) or LPa (p= 0.68). Spearman's rank-order correlation showed a statistically significant inverse correlation between TSH level and LPa (rho= -0.146, p=0.012) and identified a correlation between TSH level and HDL (rho= 0.146, p=0.013). Framingham risk index was not statistically different between patients with SCH and normals (p=0.33). **SCH was not associated with an extensive array of CHD risk factors in our population. Our findings support following the current endocrinology guidelines, offering thyroid replacement for SCH only when symptoms of hypothyroidism are clinically compelling. In our Nurse Practitioner managed CPP, the diagnosis of SCH does not appear to warrant thyroid replacement therapy for cardiovascular benefit but should be carefully considered for each patient's circumstances.**

4. To examine the relationship between stress reduction and sleep improvement, we measured changes in perceived stress and its correlation with sleep quality in a longitudinal outcomes study. Of 66 consecutive graduates (mean age 59.6+11.6, 28 men) reduced their PSS 3.1±5.8 points and improved their PSQI 1.2±2.9 points. Fifty subjects were able to reduce their PSS by a mean of 5.5±4.5 points accompanied by improvements in PSQI (1.9±3.0 points), Lp-PLA2 (41.6±53.8 mg/dL), glucose (2.0±9.1 mg/dL), insulin (2.2±7.0 ug/dL) and HOMA (0.04±1.69). The other 16 subjects showed increases in PSS of 4.3±2.0, p<0.001 accompanied by worsening PSQI (0.27±2.49, p=0.02), Lp-PLA2 (21.7±65.5, p=0.02), glucose (2.8±11.0, p=0.08), insulin (1.4±6.1, p=0.07) and HOMA (0.49±1.51, p=0.04). **Reductions in perceived stress correlate significantly with improvements in sleep quality. Improvements in perceived stress and sleep quality are accompanied by improvements in cardiovascular risk markers including glucose metabolism and lipids. Our findings underscore the importance and value of utilizing stress management techniques as a teachable sleep improvement intervention.**

5. We hypothesized that while the Berlin Questionnaire successfully screens for patients with sleep apnea and detects a population at higher risk for CV disease, the survey tool may also identify a population at risk for traits that compromise adherence to therapies for CV risk improvement. There were no differences between groups for other traditional risk factors such as total cholesterol (p=0.43), LDL cholesterol (p=0.08), lipoprotein (a) (p=0.63), and CRP (p=0.12). **The Berlin Questionnaire does help identify a population of subjects at**

greater risk for CV disease as well as traits of anxiety and perceived stress that may diminish the patients' ability to comply with CV risk reduction therapy. An important implication is that sleep apnea therapy should be implemented not only to improve CV risk but to aid the management of anxiety trait and perceived stress trait with the goal of enhancing adherence to behavior change and improving overall quality of life.

6. We sought to compare the performance of the Framingham Risk Score (FRS) with a CV Score previously validated by the Integrative Cardiac Health Project (ICHP) in a cohort of subjects with known subclinical atherosclerotic disease by abnormal carotid intima-media thickness (CIMT) measurement. In 93 consecutive subjects, mean age was 53.1±11.13 yrs, 59% women, 47% African-American, 46% Caucasian, 3% Latina, 1% other. Diagnosis of diabetes was present in 13% of subjects. Means: BMI=31.2±5.3 kg/m^2 , WC=100.2±13.6 cm, fasting glc=99.1±35.9 mg/dL, insulin 15.6±14.4 ug/dL, Tchol=194.9±42.3 mg/dL, LDL 114.5±34.0 mg/dL, HDL 56.2±18.5 mg/dL, TG 117.3±66.3 mg/dL, Lp(a)=86.5±92.3 mg/dL, CRP 0.4±0.6 mg/dL. By FRS, 12 (14%) subjects scored high risk, 11 (12%) scored medium and 70 (75%) scored low risk. By ICHP CV Risk Score, 4 (36%) of the FRS medium upscored to high risk and 47 (67%) of the FRS low upscored to medium risk. In total, 63% upscored to an appropriately higher risk category by using the ICHP CV Risk Score**. In a population with documented subclinical atherosclerosis and unremarkable conventional risk factor profiles, the ICHP CV Risk Score appeared to be more sensitive in identifying subjects at risk. The ICHP CV Risk Score may be a more discerning tool to guide risk reduction therapy in a prevention program.**

7. We hypothesized that important racial differences exist in subjects enrolling in a heart health program. Of 350 consecutive subjects (mean age 55.1 yrs, 28% men), there were 133 white (38%), 105 black (30%), 90 mixed race/undeclared, 14 Latino, and 8 others. For this analysis, only white and black subjects were considered. White subjects were somewhat older (57.4±12.6 yr vs 52.1±12.4, p=0.001) and included more men (47% v 34%, p=0.04). BMI was similar between groups (29.5±5.1 kg/m^2 vs 30.6±6.6, p=0.18). White subjects had lower perceived stress (PSS=19.4±9.6 vs 23.6±6.8, p<0.001), better sleep quality (PSQI=6.1±4.1 vs 7.1±3.9, p=0.05), and less daytime sleepiness (ESS=8.0±4.9 vs 9.8±5.0, p=0.01). White subjects tended to have less fatigue (FVAS=3.9±2.5 vs 4.5±2.4, p=0.08) with longer sleep duration (20 min longer per night, p=0.07). However, there was no difference in sleep latency (24.4 min vs 23.0, p=0.85) or likelihood for sleep apnea (Berlin positive 44% vs 51%, p=0.40). **There are important differences in levels of perceived stress, sleep quality and daytime sleepiness between white and black subjects in our program. These differences deserve explanation and may be valuable in designing interventions tailored for specific groups.**

Sub Task #10.1 Continue "Validation of the ICHP Cardiovascular Risk Score" protocol.

Data previously collected on patients enrolled in the Prospective Army Coronary Calcium (PACC) and PACC Rescan projects were reviewed. Specific information was gathered and analyzed to give each patient a CV disease risk score according to a formula developed by the ICHP. This ICHP formula uses the Framingham model of risk prediction and adds historical factors and biochemical markers to produce a novel score predictive of CV disease risk in military beneficiaries. The goal of the study was to validate the utility of this novel ICHP scoring system by comparing the predicted risk with outcomes in this well characterized population. The primary objective of the project was to validate the predictive utility and accuracy of the ICHP CV risk score (or ICHP score). Specifically, the goals: a) to determine if the ICHP score correlates with cross-sectional prevalence of coronary calcium as measured in the PACC project and b) with the development of CHD events such as angina, myocardial infarction, or need for CV intervention such as coronary stenting, angioplasty, or bypass surgery. A third goal: c) to determine the correlation of the ICHP score with coronary calcium progression as measured in the PACC rescan project.

Status: After statistical analysis of data from the PACC project, ICHP score performed successfully in the linear model with a coefficient of 0.003 (p=0.004), indicating that an increase of one point in ICHP score was associated with increasing CIMT 0.3%. In the logistic model, the odds ratio for the ICHP score was 1.04 (p=0.01), signifying that a one point increase in ICHP score increases odds by 4% of having a top quartile "atherosclerosis score". In conclusion, incorporating novel risk factors such as those proposed in the ICHP score and considering the value of family history may significantly improve the predictive accuracy of CVD risk assessment and may reveal appropriate targets for therapeutic intervention.

QA of data to ensure integrity of data collected according to ICHP CV Score parameters completed. We further utilized the principles and equations of the ICHP CV Score to analyze new data sets to demonstrate the clinical and practical use of this validated scoring tool as seen in BATTLE Study sub analysis. This sub analysis demonstrated that the ICHP Risk Score dramatically improves CV disease risk prediction in this cohort of women with subclinical atherosclerosis. These findings emphasize the need for improved CV disease risk identification in women. Family history and other novel risk factors add predictive value to current risk models and identify potential therapeutic targets.

In the past year, the following abstracts were *presented* as posters at national meetings (See Appendix A):

- Kashani M, Eliasson A, Bailey K, Vernalis M. Novel tool improves CV risk stratification and guides therapy. Quality of Care and Outcomes Research in CV Disease and Stroke, May 2011: Washington DC.
- Walizer E, Kashani M, Eliasson A, Vernalis M. Integrative cardiac health project risk score improves cardiovascular risk assessment in women with subclinical atherosclerosis**.** PCNA, Mar 2011: Orlando, FL. (Abstract was selected as the 1st place winning abstract in Data-Base Research category and published in the *Journal of Cardiovascular Nursing*, Jul 2011.)

In the past year, the following abstract was *presented* as a podium presentation at a national meeting (See Appendix A):

- Modlin R, Walizer E, Kashani M, Eliasson A, Vernalis M. Integrative Cardiac Health Project Risk Score Improves Cardiovascular Risk Assessment in Women with Subclinical Atherosclerosis**.** Army ACP, 2010: Bethesda, MD.

- Analyzed data set with new approaches utilizing skills of statistician at Department of Clinical Investigation.
- Manuscript preparation underway
- Subgroup analysis underway for young adults and women.
- Analysis to continue with data set from ZENITH study.

Sub Task #10.2 Continue "Caregivers Optimizing Readiness thru Prevention Strategies" programs (subgroup of CPP).

This proposal provides a comprehensive health program for the WRAMC nursing staff, including prescriptions for therapeutic lifestyle change.

Project participants complete questionnaires and lab tests to evaluate individual CV risk and identify emotional/behavioral triggers of stress. A dedicated workshop at ICHP delivers comprehensive instruction on diet, exercise, sleep, and stress management. Follow-up over 12 weeks includes facilitated group support sessions, coaching on coping skills, tension tamer techniques, and scheduled group exercise sessions. Participants are engaged by telephone and email to track progress and deliver pertinent instruction and encouragement. At the end of the 12-week program, measures are repeated to determine progress in stress reduction and changes in the CV health profile. Subsequently, participants continue to be engaged by telehealth and those who report setbacks are offered re-enrollment in the program. Data gathered on participants undergo dynamic statistical modeling to yield predictive information on

best lifestyle change strategies to employ for future participants. This dynamic statistical modeling will provide a more precise intervention strategy for incoming participants and allow for improved outcomes, greater efficiencies, and cost savings.

Status: Future pilots will be conducted pending funding. This task will be removed from future quarterly reports until funding is available for future pilots.

Sub Task #10.4 Initiate ultrapersonalized CPP Pilot to determine if targeted allocation of resources (guided by CV risk score stratification and tailored by actigraphy/survey results) may yield a cost-effective preventive care model.

Status: Currently in progress under CPP task. Reported under Task #10 (CPP).

Sub Task #10.5: Initiate "Digital Thermal Monitoring of Vascular Function" in an Integrative Cardiovascular Prevention Program (CPP) – renamed to ZENITH (randomiZed Evaluation of a Novel comprehensIve prevention program on aTHerosclerosis progression) Trial.

Status: Project previously described and will be spearheaded by WRAMC Cardiology Service. ICHP staff will participate. Further discussions for protocol development have continued this quarter.

The purpose of this single-center study, prospective, randomized, controlled, interventional trial at the Walter Reed National Military Medical Center (WRNMMC) is to investigate the impact of an innovative CVD risk factor assessment and prevention program, Integrative Cardiac Health Project-Cardiovascular Prevention Program (ICHP-CPP), on vascular health, atherosclerosis progression and left-ventricular relaxation (diastolic function) among patients with increased lifetime CVD risk, but low short term CVD risk (according to the Framingham Risk Score, FRS) compared to patients receiving usual care.

The aim of this study is to compare the impact of an innovative CVD prevention program, among patients at high lifetime risk for CVD events, but relatively low estimated short-term CVD risk as measured by the commonly employed Framingham Risk Score (FRS), on the atherosclerotic process as measured using DTM and CIMT. Ultimately, the question asks what is the impact of an integrative lifestyle change program (added to best medical practices) on the early surrogate markers of CVD.

Sub Task #10.6: Validation of CPP model of preventive care utilizing Coronary CT Angiography to measure Plaque Volume changes resulting from an integrative prevention program (protocol title to be determined).
Status: Will be conducted in subgroup of ZENITH Trial (Sub Task #10.5) population. Report under Sub Task #10.5 (ZENITH) in future.

Task #11: Continue the "Cardiovascular Risk Assessment and Prevention Program (CRC)".

Status: Study is currently ongoing.

Background:
This program is being established as a platform to address the unique needs of retired military beneficiaries at risk for CV disease. The program mirrors the Cardiac Prevention Program (CPP) designed and established by the ICHP at WRAMC. It includes conventional and novel CV risk profiling and tailored, personalized behavioral recommendations for primary or secondary prevention by an integrative team of providers comprised of a nurse case manager, psychologist, nurse practitioners, dietitians, stress management instructors, and exercise physiologists. Validated tools to screen for and measure CV risk, stress, sleep health,

compliance with dietary recommendations and exercise are standard of care. The program is an adjunct to the best medical practices provided by their primary care provider.

Phase I of the program involves each participant undergoing a comprehensive health risk assessment that is completed by a physician, followed by a four- hour “Pearls for your Heart” workshop and participants then schedule individual appointments with each modality specialist to receive education and counseling in nutrition, exercise, stress management and mind/body health. These are monthly appointments to be completed over a 4-6 month period.

Phase II of the program begins after the completion of the healthy lifestyle intervention (Phase I). During this phase each participant will again meet with the physician. During this appointment the physician will prepare the participants for the next phase and give them strategies for maintaining success on their own. The second phase of the Program provides additional reinforcement through monthly phone calls with an integrative health coach. Participants will remain in Phase 2 for five years, during which time they will come to the center for re-assessments every six months.

Subject Enrollment and Demographics:

Total subject enrollment in the CRC is 132 participants; currently 114 participants remain active in the study. Of the total participants, 70 were randomized to the intervention arm of the study (currently 60 participants remain active) and 62 participants were randomized to the control arm (54 remain active). Demographic characteristics of participants are: average age 61.8 years, 56% female, 25% veterans or the spouse of a veteran, and 23% with diagnosed coronary heart disease.

In the last quarter (May 2011- August 2011) there were a total of 417 participant visits including periodic follow up phone calls to participants enrolled in the intervention arm of the study and 44 visits by participants enrolled in the control arm of the study.

In the past quarter, blood was processed for 60 participants of the CRC program, resulting in approximately 1141 aliquots as detailed below. Participants were: 20 baseline intervention, 9 baseline control, 14 one year intervention, 8 one year control, and 9 intervention complete (4-6 month time point).

Participant-time points	60
PAXGene Tubes	65
RBCs	126

Plasma samples		Serum samples	
NMR lipids	63	Adiponectin	65
Leptin	63	Serum amyloid A	65
CRP	63	Vitamin D	65
Resistin	63	Lp(a)	65
Insulin	62	Extra Serum	255
Extra plasma	186		

From November 2010 to present, blood was collected from 111 participants at 147 time points for the CRC program. Approximately 2,805 sample aliquots were made as summarized below:

Participant-time points	147
PAXGene Tubes	158
RBCs	310

Plasma samples		Serum samples	
NMR lipids	156	Adiponectin	159

Leptin	156	Serum amyloid A	159
CRP	156	Vitamin D	159
Resistin	156	Lp(a)	159
Insulin	155	Extra Serum	627
Extra plasma	453		

Outcome Data

The intervention cohorts have shown change in the desired direction for virtually all of the measured coronary artery disease (CAD) risk factors over the initial 4-6 month period (Table 13A). Measures of obesity including weight and BMI declined ~3.5%. Levels of total cholesterol were reduced by ~8%, LDL cholesterol was lowered by 9% and triglycerides dropped by 20%. Systolic and diastolic blood pressure decreased by nearly 7%. Measures of carotid intimal-medial thickness (CIMT) also were significantly lower after the intervention phase. In addition, psychometric measures also significantly decreased, specifically, depression by 28% and hostility by 15%. Similarly, sleep quality improved by 25%. This data demonstrates that lifestyle change programs may be important for primary prevention in individuals with diagnosed CAD and those at increased risk of disease.

Results from the first long-term follow up time point are shown in Table 13B. Over the course of approximately 8-10 months, weight, BMI, diastolic blood pressure, maximum CIMT, all maintained statistical significance proving that the positive improvements in these traditional risk factors for CAD can be maintained over a longer period of time. In addition, at this time point, HgbA1c, a marker of blood sugar levels over a three month period, decreased significantly.

In Table 13C, results for the 16 month follow up time point are shown. Weight, BMI, CIMT and HgbA1c continue to maintain statistical significance. Although all variables continue to trend in the desirable direction, perhaps over time when more participants reach this designated time point and a larger sample size is achieved other markers that initially showed significance will again become statistically significant.

Table 13A. Comparison of "Baseline" to "Intervention Complete" (4-6 months) data for participants in the intervention arm of the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Intervention Complete Value (SD)	Average Change	P-Value
Weight (lbs.)	34	195.48 (43.8)	189.34 (39.2)	-6.1	<0.0001
Body Mass Index	34	30.63 (5.3)	29.64 (5.0)	-1.0	<0.001
Total Cholesterol (mg/dl)	32	191.19 (40.8)	175.66 (35.5)	-15.5	<0.01
High Density Lipids (mg/dl)	32	52.66 (14.2)	51.47 (10.4)	-1.2	0.4176
Low Density Lipids (mg/dl)	32	112.72 (32.4)	102.50 (29.4)	-10.2	<0.05
Triglycerides (mg/dl)	32	134.66 (69.9)	108.31 (47.2)	-26.3	<0.01
Systolic Blood Pressure	33	133.33 (18.8)	125.39 (15.6)	-7.9	<0.01
Diastolic Blood Pressure	33	79.88 (9.4)	75.03 (7.9)	-4.8	<0.01
Depression Scale [CES-D]	26	10.50 (9.6)	7.58 (6.2)	-2.9	<0.05
Hostility Scale [Cook-Medley]	26	6.88 (4.2)	5.88 (3.3)	-1.0	<0.05
Perceived Stress Scale [PSS]	26	12.62 (6.3)	10.92 (5.0)	-1.7	0.1667
Avg. CCA/Mean IMT	33	0.855 (0.1292)	0.768 (0.1249)	-0.087	<0.00001
Avg. CCA / Max IMT	33	0.988 (0.1549)	0.878 (0.1288)	-0.1	<0.00001
Fasting Glucose (mg/dl)	33	100 (13.7)	103 (21.1)	3.5	0.2834
HgbA1c	32	6.1 (1.01)	6.0 (1.03)	-0.1	0.3093
Cortisol	32	12.1 (4.20)	14.3 (4.20)	2.3	<0.01
TSH	32	2.13 (1.274)	1.95 (1.290)	-0.2	0.3500
Epworth Sleepiness Scale (0 to 24)	26	8 (4.4)	7 (4.0)	-0.5	0.3742
Pittsburgh Sleep Quality Index (0-21)	25	8 (5.1)	6 (3.5)	-1.5	<0.05

Table 13B. Change in outcome variables after 10 months for participants in the intervention arm of the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average 10 month value (SD)	Average Change	P-Value
Weight (lbs.)	17	192.51 (47.3)	184.09 (40.9)	-8.4	<0.01
Body Mass Index	17	29.72 (4.3)	28.40 (3.5)	-1.3	<0.01
Total Cholesterol (mg/dl)	17	185.06 (49.7)	169.82 (42.3)	-15.2	0.1212
High Density Lipids (mg/dl)	17	52.88 (9.9)	51.35 (10.7)	-1.5	0.4564
Low Density Lipids (mg/dl)	17	109.24 (38.8)	96.18 (33.3)	-13.1	0.1414
Triglycerides (mg/dl)	17	126.06 (62.5)	111.00 (55.6)	-15.1	0.0893
Systolic Blood Pressure	17	134.47 (22.7)	130.82 (19.2)	-3.6	0.4204
Diastolic Blood Pressure	17	79.29 (11.2)	72.00 (10.2)	-7.3	<0.01
Depression Scale [CES-D]	17	10.12 (10.3)	8.94 (11.7)	-1.2	0.5804
Hostility Scale [Cook-Medley]	17	7.18 (4.2)	6.41 (3.7)	-0.8	0.2939
Perceived Stress Scale [PSS]	17	12.00 (7.4)	11.59 (8.2)	-0.4	0.7964
Avg. CCA/Mean IMT	17	0.854 (0.1170)	0.812 (0.1304)	-0.042	0.1077
Avg. CCA / Max IMT	17	0.981 (0.1227)	0.920 (0.1371)	-0.1	<0.05
Fasting Glucose (mg/dl)	17	99 (12.5)	96 (13.8)	-2.7	0.5203
HgbA1c	17	6.1 (1.15)	5.7 (0.96)	-0.4	<0.01
Cortisol	17	13.1 (4.34)	13.8 (2.62)	0.7	0.4865
TSH	17	2.16 (1.268)	2.11 (1.371)	-0.1	0.8052
Epworth Sleepiness Scale (0 to 24)	17	7 (4.5)	6 (4.0)	-0.6	0.1869
Pittsburgh Sleep Quality Index (0-21)	17	8 (4.8)	6 (3.5)	-1.4	0.1328

Table 13C. Change in outcome variables after 16 months for participants in the intervention arm of the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average 10 month value (SD)	Average Change	P-Value
Weight (lbs.)	13	191.54 (43.3)	182.88 (41.0)	-8.7	<0.01
Body Mass Index	13	29.72 (4.3)	28.46 (3.7)	-1.3	<0.05
Total Cholesterol (mg/dl)	13	180.23 (47.9)	170.00 (40.2)	-10.2	0.2531
High Density Lipids (mg/dl)	13	52.08 (11.2)	50.08 (9.7)	-2.0	0.4296
Low Density Lipids (mg/dl)	13	106.77 (36.7)	98.38 (27.8)	-8.4	0.1671
Triglycerides (mg/dl)	13	113.62 (54.8)	107.54 (56.2)	-6.1	0.5624
Systolic Blood Pressure	13	129.69 (14.1)	123.54 (14.7)	-6.2	0.1225
Diastolic Blood Pressure	13	78.46 (12.1)	74.92 (6.9)	-3.5	0.2190
Depression Scale [CES-D]	9	10.78 (12.6)	10.11 (9.8)	-0.7	0.8078
Hostility Scale [Cook-Medley]	9	7.67 (4.4)	7.67 (5.0)	0.0	1.0000
Perceived Stress Scale [PSS]	9	12.00 (8.6)	11.44 (7.4)	-0.6	0.7209
Avg. CCA/Mean IMT	13	0.851 (0.1196)	0.736 (0.1370)	-0.115	<0.00001
Avg. CCA / Max IMT	13	0.981 (0.1197)	0.855 (0.1551)	-0.1	<0.0001
Fasting Glucose (mg/dl)	13	101 (12.7)	103 (25.9)	2.8	0.6802
HgbA1c	13	6.3 (1.20)	6.0 (1.32)	-0.3	<0.05
Cortisol	13	13.2 (4.78)	14.5 (3.27)	1.2	0.3948
TSH	13	2.31 (1.336)	2.60 (1.719)	0.3	0.1541
Epworth Sleepiness Scale (0 to 24)	9	6 (4.2)	6 (4.4)	-0.6	0.4008
Pittsburgh Sleep Quality Index (0-21)	9	8 (4.5)	6 (3.1)	-1.9	0.1050

In subjects randomized to the control arm of the study, who do not participate in the lifestyle change intervention, many risk factors did not show significant changes after 6 months in the study (Table 14A). Total cholesterol, LDL cholesterol, depression and carotid IMT measures did

decrease significantly, but this may be a statistical anomaly attributable to previous participation in another lifestyle change program by some of the control participants who could be continuing to practice previously learned healthy lifestyle changes. At one year (Table 14B), no variables remained significant except for CIMT and HgbA1c but these positive changes may be a statistical anomaly attributable to small sample size and wide inter-individual variability. No control subjects have yet reached any further time points.

Table 14A. Change in outcome variables after 6 months for participants in the control arm of the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Waiting Period Complete Value (SD)	Average Change	P-Value
Weight (lbs.)	26	195.27 (43.6)	194.43 (42.9)	-0.8	0.5897
Body Mass Index	26	31.49 (7.2)	31.72 (7.1)	0.2	0.5826
Total Cholesterol (mg/dl)	28	199.96 (33.1)	186.86 (34.0)	-13.1	<0.05
High Density Lipids (mg/dl)	28	55.46 (17.6)	53.86 (13.3)	-1.6	0.3729
Low Density Lipids (mg/dl)	28	119.46 (27.5)	107.18 (27.4)	-12.3	<0.05
Triglycerides (mg/dl)	28	131.86 (66.4)	129.07 (69.8)	-2.8	0.8109
Systolic Blood Pressure	28	130.14 (16.6)	130.93 (20.7)	0.8	0.8345
Diastolic Blood Pressure	28	78.50 (10.3)	76.36 (11.2)	-2.1	0.2198
Depression Scale [CES-D]	27	11.11 (9.8)	9.11 (9.4)	-2.0	<0.05
Hostility Scale [Cook-Medley]	27	7.26 (5.2)	7.00 (5.5)	-0.3	0.5508
Perceived Stress Scale [PSS]	27	12.70 (8.1)	11.48 (8.0)	-1.2	0.1533
Avg. CCA / Mean IMT	28	0.909 (0.1600)	0.840 (0.1557)	-0.069	<0.001
Avg. CCA / Max IMT	28	1.044 (0.1743)	0.952 (0.1699)	-0.1	<0.0001
Fasting Glucose (mg/dl)	28	120 (49.7)	125 (57.7)	4.6	0.4535
HgbA1c	27	6.6 (1.78)	6.5 (1.38)	0.0	0.8278
Cortisol	28	12.4 (4.33)	14.2 (3.99)	1.9	<0.05
TSH	27	1.86 (1.128)	1.93 (1.088)	0.1	0.7094
Epworth Sleepiness Scale (0 to 24)	27	9 (4.4)	8 (3.9)	-1.2	0.1349
Pittsburgh Sleep Quality Index (0-21)	27	7 (4.1)	6 (3.6)	-0.3	0.6642

Table 14B. Change in outcome variables after one year for participants in the control arm of the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Waiting Period Complete Value (SD)	Average Change	P-Value
Weight (lbs.)	16	203.74 (41.6)	201.70 (41.4)	-2.0	0.2769
Body Mass Index	16	33.42 (5.6)	33.03 (5.9)	-0.4	0.2458
Total Cholesterol (mg/dl)	16	208.50 (37.0)	197.31 (40.8)	-11.2	0.0955
High Density Lipids (mg/dl)	16	56.69 (20.6)	58.44 (25.0)	1.8	0.7225
Low Density Lipids (mg/dl)	16	125.75 (29.7)	117.50 (33.8)	-8.3	0.2505
Triglycerides (mg/dl)	16	141.88 (75.5)	132.31 (59.8)	-9.6	0.5299
Systolic Blood Pressure	16	137.25 (16.6)	139.25 (31.6)	2.0	0.7638
Diastolic Blood Pressure	16	84.13 (8.7)	79.63 (12.1)	-4.5	0.0952
Depression Scale [CES-D]	17	10.71 (10.0)	10.41 (10.6)	-0.3	0.7821
Hostility Scale [Cook-Medley]	17	7.65 (6.0)	7.18 (5.0)	-0.5	0.4539
Perceived Stress Scale [PSS]	17	12.47 (8.0)	12.06 (8.2)	-0.4	0.7644
Avg. CCA / Mean IMT	16	0.941 (0.1720)	0.865 (0.1480)	-0.076	<0.01
Avg. CCA / Max IMT	16	1.066 (0.1881)	1.007 (0.1708)	-0.1	<0.05
Fasting Glucose (mg/dl)	16	120 (47.9)	113 (35.3)	-7.3	0.2890

HgbA1c	16	6.6 (1.43)	6.2 (0.97)	-0.4	<0.05
Cortisol	16	12.6 (4.67)	14.5 (4.08)	1.9	0.0904
TSH	16	1.62 (0.761)	2.17 (0.899)	0.6	<0.05
Epworth Sleepiness Scale (0 to 24)	17	9 (4.8)	8 (4.5)	-0.5	0.4716
Pittsburgh Sleep Quality Index (0-21)	17	7 (4.6)	7 (4.5)	0.1	0.8527

Adverse Events

All adverse events are submitted to and adjudicated by the Windber Medical Center Institutional Review Board and TATRC after review by both the Principal Investigator and Medical Monitor. There were no adverse events in either arm of the study during the last quarter. To date there have been a total of 8 adverse events in both the intervention and control arms of the study, all deemed serious events, not related and not expected. A serious event is defined as occurring at any dose or intervention level that results in any of the following outcomes: (1) results in death, (2) a threat to life, (3) inpatient hospitalization or prolongation of existing hospitalization, (4) persistent or significant disability or incapacity, (5) causes cancer, (6) is an overdose, or (7) any medical event that requires treatment to prevent one of the medical outcomes listed above. Therefore, all 16 events were considered serious due to inpatient hospitalizations. There were 7 non-cardiac and 1 cardiac adverse events in the intervention arm of the study. No deaths occurred and none of these adverse events were deemed to be study related. There were 5 non-cardiac and 3 cardiac adverse events in the control arm of the study. No deaths occurred and none of these adverse events were deemed to be study related.

NMR Lipid Panel and Biomarkers

During this period, we received data on the following variables for CRC participants:

NMR lipid panel	100
CRP	215
Leptin	209
Lipoprotein (a)	98
Vitamin D	128
Insulin	101
Adiponectin	95
Serum Amyloid	97
Resistin	100

Some results received during this period were from blood samples drawn prior to November 2010. Due to the untimely passing of our database manager and statistician, we were not able to summarize these results at this time.

Task #11a: Continue "Stress Therapy Empowering Prevention (STEP)" component to the Cardiovascular Risk Assessment program outlined in Task #11.

Status: Study is currently ongoing.

In the past year, the following abstracts were *presented* as posters at a national meeting (See Appendix A):

- Burke A, Haberkorn J, Lechak F, Sullivan J, Vizza J, Vernalis MN, Ellsworth DL. Stress Therapy Empowers Prevention (STEP): A healthy-lifestyle program for breast cancer patients. National Consortium of Breast Centers, 21st Annual Interdisciplinary Breast Center Conference, March 2011: Las Vegas, NV.

- Ellsworth DL, Patney HL, Burke A, Haberkorn J, Lechak F, Sullivan J, Vizza J, Neatrour DM, Vernalis MN. Improvement in cardiovascular risk factors in breast cancer patients participating in the Stress Therapy Empowers Prevention (STEP) program. National Consortium of Breast Centers, 21st Annual Interdisciplinary Breast Center Conference, March 2011: Las Vegas, NV.

Background:

This is a collaborative study involving researchers from Windber Research Institute and Walter Reed Army Medical Center and is modeled after the Caretakers Optimizing Readiness through Preventive Strategies (CORPS), designed by the Integrative Cardiac Health Program (ICHP) at Walter Reed Army Medical Center (WRAMC), except that it targets participants with chronic disease. The purpose of this task is to determine the degree of stress, sleep disturbance, and cardiovascular disease risk in patients who have been diagnosed with breast cancer or are at high risk of developing breast disease.

In the first part of the intervention, patients will be randomized to a 12 week Healthy Lifestyle intervention group or a non-intervention group. During this phase, each intervention participant undergo a comprehensive health risk assessment that is completed by a physician, followed by mandatory attendance to on-site group sessions in which they will participate in 1 hour of stress management, 30 minutes of nutrition education every week, and 30 minutes of exercise alternated with 30 minutes of mind/body health every other week. In addition, the nurse will provide educational lectures on various health topics during 4 sessions. After completing Phase I, patients will participate in a five year healthy lifestyle intervention or control group.

During phase II each intervention participant will again meet with the physician. During this appointment the physician will prepare the participants for the next phase and give them strategies for maintaining success on their own. The second phase of the program provides additional reinforcement through monthly phone calls with an integrative health coach. Participants will remain in Phase II for five years, during which time they will come to the center for re-assessments every six months.

We hypothesize that the 12 week healthy lifestyle interventions will significantly reduce stress, sleep disturbances, and cardiovascular risk in patients at risk for, or already diagnosed with, breast cancer.

Subject Enrollment and Demographics:

Total subject enrollment is 18; currently 16 participants remain active in the study. All 18 of these participants are enrolled in the intervention arm of the study. Demographic characteristics of participants are: average age 65.6 years, 28% veterans or the spouse of a veteran, 6% have diagnosed coronary heart disease, and 61% have diagnosed breast cancer.

In the last quarter (May 2011- August 2011) there were a total of 31 participant visits including periodic follow up phone calls made to participants.

During this quarter, blood was processed for the second cohort of the STEP program from 6 participants at the 1-year examination. Approximately 123 aliquots were made as summarized by the following:

Individual Participants	6
PAXGene Tubes	7
RBCs	14

Plasma samples		Serum samples	
NMR lipids	7	Adiponectin	7
Leptin	7	Estradiol	7
Resistin	7	HER2	7
TNFα	7	Serum amyloid A	7
Insulin	7	Vitamin D	7
CRP	7	Lp(a)	7
Extra plasma	10	Extra serum	15

From November 2010 to present, blood was collected and processed for 14 participant-time points for the STEP program. Approximately 294 sample aliquots were made as summarized below:

Individual Participants	14
PAXGene Tubes	16
RBCs	32

Plasma samples		Serum samples	
NMR lipids	16	Adiponectin	16
Leptin	16	Estradiol	16
Resistin	16	HER2	16
TNFα	16	Serum amyloid A	16
Insulin	16	Vitamin D	16
CRP	16	Lp(a)	16
Extra plasma	33	Extra serum	37

Outcomes Data:

Participants thus far are showing change in the desired direction for most of the measured coronary artery disease (CAD) risk factors over the initial 12 week period (Table 15A). Results from the first year follow up time point are show in Table 15B. Only one cohort has reached the 18 month time point (Table 15C) and no participants have been enrolled into the control arm of the study. Lack of statistically significant levels of improvement in some measures may be attributable to small sample size and wide variability in some measures.

Table 15A. Comparison of baseline to Week 12 data for participants in the STEP Program

Category / Metrics	N	Average Baseline Value (SD)	Average Week 12 Value (SD)	Average Change	P Value
Weight (lbs.)	16	182.57 (35.9)	179.30 (33.0)	-3.3	<0.01
Body Mass Index	16	32.83 (6.3)	32.04 (5.9)	-0.8	<0.01
Total Cholesterol (mg/dl)	16	198.38 (36.4)	196.69 (44.2)	-1.7	0.7954
High Density Lipids (mg/dl)	16	54.44 (12.4)	52.25 (12.8)	-2.2	0.0928
Low Density Lipids (mg/dl)	16	114.50 (28.7)	118.63 (38.4)	4.1	0.5290
Triglycerides (mg/dl)	16	155.13 (90.6)	132.81 (73.4)	-22.3	0.0926
Systolic Blood Pressure	16	134.75 (18.8)	124.50 (14.1)	-10.3	0.0763
Diastolic Blood Pressure	16	80.63 (11.3)	73.75 (8.1)	-6.9	<0.05
Depression Scale [CES-D]	16	15.31 (10.2)	11.44 (10.4)	-3.9	0.0914
Hostility Scale [Cook-Medley]	16	7.06 (4.4)	5.25 (3.3)	-1.8	0.0720
Perceived Stress Scale [PSS]	16	17.00 (7.2)	12.88 (6.5)	-4.1	<0.05
Avg. CCA/Mean IMT	16	0.735 (0.1488)	0.810 (0.1677)	0.075	<0.01
Avg. CCA / Max IMT	16	0.865 (0.1556)	0.928 (0.2046)	0.1	<0.05
Fasting Glucose (mg/dl)	16	107 (28.8)	109 (25.7)	2.4	0.6604
HgbA1c	16	6.3 (0.87)	6.5 (0.77)	0.2	0.3545
Cortisol	16	12.8 (3.83)	16.5 (5.44)	3.7	0.0507
TSH	16	1.71 (1.342)	2.07 (1.674)	0.4	0.2887
Epworth Sleepiness Scale (0 to 24)	16	9 (4.5)	8 (4.2)	-0.9	0.4320
Pittsburgh Sleep Quality Index (0-21)	16	10 (4.8)	8 (4.4)	-2.5	0.0512

Table 15B. Comparison of baseline to Year 1 data for participants in the STEP program

Category / Metrics	N	Average Baseline Value (SD)	Average Year 1 Value (SD)	Average Change	P Value
Weight (lbs.)	14	180.49 (35.5)	177.30 (32.7)	-3.2	<0.05
Body Mass Index	14	32.49 (6.4)	31.66 (6.0)	-0.8	<0.05
Total Cholesterol (mg/dl)	14	201.07 (37.3)	200.21 (45.8)	-0.9	0.9083
High Density Lipids (mg/dl)	14	54.64 (13.1)	52.14 (13.7)	-2.5	0.0715
Low Density Lipids (mg/dl)	14	116.79 (30.0)	121.57 (40.2)	4.8	0.5252
Triglycerides (mg/dl)	14	157.21 (95.4)	136.71 (76.1)	-20.5	0.1721
Systolic Blood Pressure	14	134.00 (18.4)	125.14 (15.0)	-8.9	0.1451
Diastolic Blood Pressure	14	79.57 (11.3)	72.86 (8.3)	-6.7	0.0838
Depression Scale [CES-D]	14	13.71 (9.7)	11.29 (11.0)	-2.4	0.1056
Hostility Scale [Cook-Medley]	14	6.36 (4.2)	4.79 (3.2)	-1.6	0.3330
Perceived Stress Scale [PSS]	14	16.29 (7.4)	12.71 (6.8)	-3.6	<0.05
Avg. CCA/Mean IMT	14	0.745 (0.1557)	0.826 (0.1718)	0.081	<0.01
Avg. CCA / Max IMT	14	0.879 (0.1607)	0.948 (0.2110)	0.1	<0.05
Fasting Glucose (mg/dl)	14	109 (30.6)	111 (27.4)	2.0	0.7535
HgbA1c	14	6.4 (0.91)	6.6 (0.77)	0.2	0.3356
Cortisol	14	12.4 (3.61)	16.9 (5.61)	4.5	<0.05
TSH	14	1.66 (1.419)	2.19 (1.766)	0.5	0.1559
Epworth Sleepiness Scale (0 to 24)	14	8 (4.5)	8 (4.5)	-0.6	0.6020
Pittsburgh Sleep Quality Index (0-21)	14	10 (5.1)	8 (4.6)	-2.7	0.0639

Table 15C. Comparison of baseline to 18 month data for participants in the STEP program

Category / Metrics	N	Average Baseline Value (SD)	Average Year 1 Value (SD)	Average Change	P Value
Weight (lbs.)	8	177.39 (34.5)	175.70 (35.8)	-1.7	0.5667
Body Mass Index	8	31.71 (5.9)	31.15 (5.9)	-0.6	0.3391
Total Cholesterol (mg/dl)	8	215.38 (35.7)	228.38 (58.1)	13.0	0.4383
High Density Lipids (mg/dl)	8	53.88 (13.5)	54.25 (12.4)	0.4	0.8625
Low Density Lipids (mg/dl)	8	126.00 (29.5)	139.13 (42.2)	13.1	0.2738
Triglycerides (mg/dl)	8	193.38 (113.4)	173.38 (126.2)	-20.0	0.4368
Systolic Blood Pressure	8	136.25 (23.9)	132.50 (19.1)	-3.8	0.6882
Diastolic Blood Pressure	8	81.75 (14.6)	73.50 (10.2)	-8.3	0.0764
Depression Scale [CES-D]	7	14.71 (5.8)	7.71 (5.4)	-7.0	0.0758
Hostility Scale [Cook-Medley]	7	6.29 (4.5)	5.29 (4.1)	-1.0	0.2815
Perceived Stress Scale [PSS]	7	20.00 (3.7)	10.57 (4.9)	-9.4	<0.01
Avg. CCA/Mean IMT	8	0.674 (0.0752)	0.732 (0.0800)	0.058	0.0586
Avg. CCA / Max IMT	8	0.811 (0.0914)	0.863 (0.0912)	0.1	0.1135
Fasting Glucose (mg/dl)	8	117 (36.6)	116 (43.5)	-0.5	0.9238
HgbA1c	8	6.8 (0.90)	6.8 (1.65)	0.0	0.9769
Cortisol	8	13.1 (3.61)	12.7 (4.87)	-0.4	0.8843
TSH	8	1.94 (1.732)	1.69 (1.186)	-0.2	0.5556
Epworth Sleepiness Scale (0 to 24)	7	8 (5.4)	7 (4.3)	-1.3	0.4309
Pittsburgh Sleep Quality Index (0-21)	7	13 (2.2)	8 (4.8)	-5.0	0.0589

Adverse Events:

All adverse events are submitted to and adjudicated by the Windber Medical Center Institutional Review Board and TATRC after review by both the Principal Investigator and Medical Monitor. There was one adverse event during the last quarter, the event was deemed serious, not related and unexpected due to testing that revealed terminal metastasis to the bone and adrenal

gland. To date, there have been 5 adverse events, 4 were deemed serious and 1 event was not serious. A serious event is defined as occurring at any dose or intervention level that results in any of the following outcomes: (1) results in death, (2) a threat to life, (3) inpatient hospitalization or prolongation of existing hospitalization, (4) persistent or significant disability or incapacity, (5) causes cancer, (6) is an overdose, or (7) any medical event that requires treatment to prevent one of the medical outcomes listed above. Three of the events were considered serious due to inpatient hospitalizations and one due to poor prognosis related disease progression. No deaths occurred and none of these adverse events were deemed to be study related.

Protein profiling of breast cancer patients with CVD risk factors:
Additional samples have been requested (Stage I n=19, Stage IV n=20) to increase the sample size for this technology development project, which aims to determine protein profile differences among breast cancer patients with cardiovascular risk factors. Plasma samples will again be assayed for 40 analytes that have the potential to be altered in either cancer or CVD using xMAP technology and Luminex instrumentation. Because our preliminary results showed a significant difference between Stage I and Stage IV breast cancer patients with known cardiovascular risk factors for levels of EGF, IFNγ, IL-8, IL-13, Survivin, and SFasL, we will continue this project to attain a statistically relevant sample size.

The assay design and technology for this project can also be used to further study the plasma protein profiles of patients with CVD enrolled in our programs.

Task #*12*: Continue "Defining the Genetic Basis of Heart Attack and Acute Coronary Syndromes in Military Service Women" (In collaboration with WRAMC ICHP).

This study will identify genes that affect susceptibility to heart attack in young military service personnel who have had a heart attack before the age of 55. Patients will be selected from the Department of Defense Serum Repository, which has millions of serum samples in storage. Cutting-edge technology will be used to isolate very small amounts of DNA that can be found in serum. The entire genome will then be amplified and 500,000 variations in the DNA will be tested. The ultimate objective is to identify new genes that increase risk for heart attack at an early age – such genes represent new targets for preventive or therapeutic interventions.

Status:
Institutional Review Board approval was received from Windber Medical Center on June 27, 2008. The feasibility study protocol is ready to be submitted to Department of Clinical Investigation Institutional Review Board at Walter Reed Army Medical Center. Letter of support received from DODSR. Letter of Collaborative Agreement received from HJF. Awaiting review by WRAMC for Material Transfer Agreement (MTA). Planned submission next quarter.

Based on conversations with Dr. Vernalis at WRAMC, we have revised the study protocol, which will be initiated as a feasibility study. This modification in the study design will determine the feasibility of isolating, amplifying, and genotyping quality DNA from serum samples in the Department of Defense Serum Repository (DoDSR). For this proof-of-principal study we aim to: (1) assess the quantity and quality of DNA isolated from serum samples obtained from the DoDSR, (2) conduct whole-genome amplification of the serum DNA and evaluate the resulting whole-genome amplified DNA (wgaDNA), and (3) evaluate the performance of the obtained wgaDNA on Affymetrix 6.0 SNP arrays containing 1.6 million markers. These preliminary studies will determine if we can use DoDSR wgaDNA on high-density genetic marker arrays for future studies.

The current MI in Young Military Service Members protocol will be implemented as phase II after we demonstrate that the DoDSR samples yield quality DNA for genomic studies. We plan to revise the study to obtain 1000 serum samples from the DoDSR (500 subjects, 500 controls) to examine the genomic influences on heart attack. In addition, we will include military men and

women less than 55 years of age who have had a diagnosis of myocardial infarction or other acute coronary syndrome. This study will allow us to identify genomic differences that influence early coronary events. Discovering the underlying causes of early coronary events in young individuals will likely provide important targets for new treatments to improve their care and long-term health.

During this period, we continued our research and development work on whole-genome amplification of DNA samples and large-scale genomic research on these samples.

The following 2 abstracts were submitted to the Association for Molecular Pathology and *accepted* as poster presentations for the November 2011 meeting. Abstracts will be published in the Journal of Molecular Diagnostics.

- Voeghtly L, Croft DT Jr, Deyarmin B, Vernalis MN, Shriver CD, Ellsworth DL. Utility of whole genome amplification for assessing copy number variation with high density SNP arrays from formalin-fixed paraffin embedded tissue. Association for Molecular Pathology (AMP) 2011 Annual Meeting, November 17-19, Grapevine, TX.

In this study, we examined the feasibility of assessing chromosome copy number (CN) variation using whole-genome amplification on DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tissue, as well as fresh frozen tissue, and high-density Affymetrix GeneChip® 500K SNP Mapping Arrays. We were able to show that fresh frozen tissue, even if whole-genome amplified, is useful for genome-wide SNP genotyping and determining chromosome CN variation, but large discrepancies are likely to occur when using whole-genome amplification on DNA isolated from FFPE.

-Croft DT Jr, Voeghtly L, Patney HL, Shriver CD, Vernalis MN, Ellsworth DL. Performance of whole-genome amplified DNA isolated from serum and plasma for estimating copy number variation with high density single nucleotide polymorphism arrays. Association for Molecular Pathology (AMP) 2011 Annual Meeting, November 17-19, Grapevine, TX.

Here we assessed the performance of whole-genome amplified (wga) DNA derived from stored serum/plasma for estimating chromosome CN variation on high-density single nucleotide polymorphism (SNP) arrays. Storage time and usage history did not affect DNA extraction or whole-genome amplification yields; however, samples that had been thawed and refrozen did not perform as well (73.9 $\pm$ 7.8%) as samples that had never been thawed (92.0 $\pm$ 3.3%) (P<0.001). Patterns of CN variation were highly discordant between serum/plasma wgaDNA and gDNA from the same patients, suggesting that analyses of CN variation may be compromised when using whole-genome amplified DNA from serum/plasma.

Task #*13*: Initiate "Lifestyle Education and Nutrition (LEAN) Program pilot project.
Status: Pilot conducted. Findings suggest future research study not to be feasible. Task completed. This task will be removed from future quarterly reports.

Task #14: Initiate "Defining the Genetic Basis of Heart Attack and Acute Coronary Syndromes in Military Service Women" protocol. (Task in collaboration with WRI)
Status: Remove task from SOW. Proof of principle feasibility to be conducted first – see Task #12.

Task #15: Initiate certification of ICHP as optimal healing environment
Status: The formal certification process will be delayed until the impending move to WRNMMC and new database is complete. This task will be removed from quarterly reports until certification can be pursued.

Task #16: Begin pilot study to assess the utility of direct tissue protein profiling for identifying new markers of heart disease.
Status: Study not feasible at this time.

Key Research Accomplishments

- Non-Invasive Coronary Artery Disease Reversal" (CADRe) Study Protocol
  - Final manuscript published

- Non-Invasive Coronary Artery Disease Reversal (CADRe) Follow-Up Study
  - Study completed
  - Data reconciliation and analysis in progress
  - Publication plan in progress

- Better Adherence to Therapeutic Lifestyle Change Efforts (BATTLE) Trial
  - Main Study completed; addendum data collection complete
  - Data management complete; database locked
  - Draft data tables onsite
  - Limited onsite data analyses
  - Publication plan in progress – 2 manuscripts in progress
  - 1 abstracts presented

- Dr. Dean Ornish Program for Reversing Heart Disease protocol
  - Subject enrollment over 25 cohorts is complete – 422 participants were enrolled, 339 participants graduated, 83 participants dropped out
  - Age/gender/disease status matched control group established to compare risk factor changes

- Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal
  - Subject enrollment was 374 – 166 participants in the lifestyle change program, 140 subjects serving as the control group, and 68 participants enrolled in the Sub-study
  - One abstract presented at the Nutrition, Physical Activity and Metabolism / Cardiovascular Disease Epidemiology and Prevention 2011 Scientific Sessions in Atlanta
  - Two abstracts accepted to the Association for Molecular Pathology (AMP) 2011 Annual Meeting in Dallas, TX
  - Participation in the Program reduces levels of important biochemical risk factors for CAD, such as insulin, CRP, and leptin (manuscripts in progress).
  - Fundamental molecular changes were shown to occur in Program participants – changes in gene expression occur at 12 weeks and persist at one year
    – genes involved function in defense and immune response (manuscript in progress)
    – non-diabetics with high insulin show greater gene expression changes than diabetics or non-diabetics with low insulin
    – participants with low stress show more expression changes than high-stress participants
    – individuals who successfully lose weight show greater gene expression changes than those who are unsuccessful at weight loss
  - MIF levels decrease significantly in Ornish participants compared to controls at 12 weeks, but no difference at one year; women show significant reductions in MIF levels at 12 weeks (-23%), but not men (-2.3%); changes in MIF are influenced by genotype, –29% in participants with –794CATT7 allele
  - In several studies of gene expression changes, we observed:
    --Gene expression changes restricted to non-diabetics with high insulin compared to diabetics and non-diabetics with low insulin
    --Most expression changes occur in low-stress versus high-stress group
    --Individuals most successful at weight loss showed dramatic changes in gene expression
  - Genetic variation influences risk factor response
    --16 SNPs showed evidence of an influence on triglyceride response

--3 SNPs showed differences between genotype groups
--At 4 SNPs response differed significantly by gender for one genotype

- Cardiovascular Prevention Program (CPP):
  - Minimal Risk Protocol for retrospective review of data approved.
  - 9 peer-reviewed publications and 1 manuscript from the CPP have been generated.
  - Refinement of clinical research model based on recent findings.
  - Clinical Database development with informatics architects and vendor selection underway.
  - Data management and analysis underway.
  - Publication plan in progress.
  - Integrative approach applied to specific prevention tracks for optimal impact and improved clinical outcomes for military beneficiaries.

- Caregiver Support Program: Protocol prepared and ready for submission pending grant funding.
  - Awaiting funding.

- Validation of the ICHP Cardiovascular Risk Score
  - ICHP CV Risk score revalidated with new data set of women
  - 2 abstracts presented and published

- The Cardiovascular Risk Clinic (CRC)
  - Subject enrollment is 132; 114 participants remain active

- The Stress Therapy Empowering Prevention (STEP) program
  - Two abstracts presented at the National Consortium of Breast Centers Interdisciplinary Breast Center Conference in Las Vegas

Reportable Outcomes

Manuscripts in Scientific Journals:

Kashani M, Eliasson A, Vernalis M. Stress is a modifiable risk factor for stroke prevention, *Stress: International Journal of Biology of Stress*, 2011, 19 Jun (epub ahead of print).

Marshall D, Walizer E, & Vernalis M. Effect of a One-Year Lifestyle Intervention Program on Carotid Intima Media Thickness, *Mili Med* 2011; 176(7):798-804.

Abstracts in Scientific Journals:

Eliasson A, Kashani M, Hoffman J, Vernalis M. Racial differences in perceived stress, sleep habits, and daytime symptoms*. Sleep* 2011; accepted for publication

Kashani M, Eliasson A, Bailey K, Vernalis M. Novel tool improves CV risk stratification and guides therapy. *Circ Cardiovasc Qual Outcomes* 2011; accepted for publication

Eliasson A, Kashani M, Bailey K, Vernalis M. The Berlin questionnaire identifies a population with traits inhibiting adherence. *Am J Respir Crit Care Med* 2011; 183:A1444

Walizer E, Kashani M, Eliasson A, Vernalis M. Integrative Cardiac Health Project risk score improves cardiovascular risk assessment in women with subclinical atherosclerosis. *Journal of Cardiovascular Nursing* 2011; 26(4): 265A.

Eliasson A, Kashani M, Mayhew M, Ude A, Hoffman J, Vernalis M. Reducing perceived stress improves sleep quality—a longitudinal outcomes study. *CHEST* 2010; 137:913A.

Eliasson AH, Kashani M, Mayhew M, Vernalis M. Improving sleep quality correlates with lower weight—A longitudinal outcomes study. *Sleep* 2010; 33:A378.

Kashani M, Eliasson A, Vernalis M. Prediabetics improve CV risk profile by reducing stress. *Circ Cardiovasc Qual Outcomes* 2010; 3:P58.

Eliasson AH, Kashani M, Vernalis M*. Longer sleep time confers cardiovascular health benefit. Am J Respir Crit Care Med* 2010; 181:A6524.

Kashani M, Eliasson A, Hoffman J, Vernalis M. Assessing perceived stress provides targets for stroke prevention. *Stroke* 2010; 41:e292.

Presentations:

Burke A, Haberkorn J, Lechak F, Sullivan J, Vizza J, Vernalis MN, Ellsworth DL. Stress Therapy Empowers Prevention (STEP): A healthy-lifestyle program for breast cancer patients. National Consortium of Breast Centers, 21st Annual Interdisciplinary Breast Center Conference, March 2011: Las Vegas, NV.

Ellsworth DL, Patney HL, Burke A, Haberkorn J, Lechak F, Sullivan J, Vizza J, Neatrour DM, Vernalis MN. Improvement in cardiovascular risk factors in breast cancer patients participating in the Stress Therapy Empowers Prevention (STEP) program. National Consortium of Breast Centers, 21st Annual Interdisciplinary Breast Center Conference, March 2011: Las Vegas, NV.

Ellsworth DL, Soltow QA, Kolli K, Patney HL, Jones DP, Vernalis MN. Cardiac rehabilitation involving lifestyle modification alters comprehensive plasma metabolomic profiles defined by LC-FTMS. Nutrition, Physical Activity, and Metabolism/Cardiovascular Disease Epidemiology and Prevention 2011 Scientific Sessions, March 2011: Atlanta, GA.

Modlin R, Walizer E, Kashani M, Eliasson A, Vernalis M. Integrative Cardiac Health Project Score improves CV risk assessment in women with subclinical atherosclerosis. American College of Physicians, Army Region, Nov 2010, podium presentation.

Modlin R, Kashani M, Eliasson A, Bailey K, Vernalis M. The need for cardiovascular prevention in young military service members. American College of Physicians, Army Region, Nov 2010, poster presentation.

Modlin R, Kashani M, Eliasson A, Vernalis M. Comprehensive early assessment is critical for CV prevention. American College of Physicians, Army Region, Nov 2010, podium presentation.

Eliasson A, Kashani M, Mayhew M, Ude A, Hoffman J, Vernalis M. Reducing perceived stress improves sleep quality—a longitudinal outcomes study. American College of Chest Physicians, Oct 2010, podium presentation.

Mayhew M, Eliasson A, Kashani M, Vernalis M. Should subclinical hypothyroidism be treated to lower cardiovascular risk? American College of Nurse Practitioners, Oct 2010: poster presentation.

Conclusions

Unhealthy lifestyle behaviors are linked to the development of CHD, as well as other chronic diseases. Projections based on combined CVD risk factor impact suggest that favorable lifestyle habits can substantially reduce CHD morbidity and mortality. We continue to demonstrate that comprehensive lifestyle interventions are remarkably efficacious in reducing CVD risk factors and, in many cases, are comparable to pharmacological interventions. Our research endeavors from this project continue to provide new information regarding strategies for improving the adoption of healthy lifestyle behaviors, the impact of lifestyle interventions on CVD risk, and the biologic mechanisms through which lifestyle changes exert their influence. Through this research, the DOD has a unique opportunity to identify and address adverse lifestyle behaviors and CVD risk factors early and make CV health a part of the military culture. A commitment to CV health could prevent cardiac events, reduce the need for costly procedures and hospitalization, improve quality of life and protect the investment of highly trained military personnel.

Appendix A
Manuscript/Abstracts

The Effect of a One-Year Lifestyle Intervention Program on Carotid Intima Media Thickness

Debra Marshall, MD; LTC Elaine Walizer, NC USA (Ret.)†; COL Marina Vernalis, MC USA (Ret.)†*

ABSTRACT This study assesses the impact of a year long lifestyle intervention program on carotid intima media thickness (CIMT) in 60 subjects, at-risk for or with coronary artery disease. We calculated mean CIMT at baseline (0.731 ± 0.151 mm) and 1 year (0.720 ± 0.129 mm), overall CIMT change and the relationship of CIMT change to the number (0–5) of achieved Heart Health Index (HHI) measures (body mass index < 25 kg/m^2, exercise ≥ 150 min/wk, blood pressure < 140/90 mm Hg, LDL-Cholesterol < 100 mg/dL, fiber intake > 25 g/d). CIMT was unchanged (−0.011 ± 0.118 mm; $p = 0.48$); however, there was a trend toward CIMT decrease (−0.025 ± 0.120 mm vs. +0.033 ± 0.102 mm; $p = 0.10$) between subjects with HHI Score ≥ 3 ($n = 45$) compared to those with an HHI Score < 3 ($n = 15$) at 1 year. These findings suggest atherosclerosis progression can be blunted with a lifestyle intervention that fully leverages nonpharmacologic approaches to cardiovascular risk reduction.

INTRODUCTION

Lifestyle interventions that include a heart healthy diet, regular physical activity, weight maintenance/reduction, smoking cessation, and stress management have been shown to prevent or reduce cardiovascular disease (CVD) risk factors.[1,2] The efficacy of these nonpharmacologic measures can be comparable to pharmacologic therapies, particularly when several health behaviors are adopted together.[3–9]

Large, controlled trials on lifestyle interventions that assess morbidity and mortality endpoints have not been performed because of their expense and difficulties in preventing carryover effects between experimental and control groups during a long-term trial. Therefore, use of surrogate markers that predict the likelihood of CVD events is becoming more accepted as an approach to improve clinical trial efficiency, duration and cost. Measurement of carotid intima media thickness (CIMT) by B-mode ultrasonography is among the imaging tools for noninvasive assessment of atherosclerosis and has been validated as a predictor of cardiovascular (CV) events in several studies.[10–13] However, studies assessing the effect of lifestyle interventions on atherosclerosis are limited.

The Coronary Artery Disease Reversal study was conducted in military health care beneficiaries, with or at-risk for coronary artery disease (CAD), to determine the feasibility and efficacy of an intensive, multicomponent lifestyle intervention. This article presents the findings of the CIMT substudy, which assessed the impact of this intervention and the number of CV health measures achieved on atherosclerosis progression over one year.

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METHODS

Study Population and Design

This is a prospective, single-arm study modeled after the Dean Ornish Program for Reversing Heart Disease[8] that was conceived to determine the feasibility and efficacy of this specific lifestyle intervention in a nonresidential military population. Volunteer subjects were self-referred military health care beneficiaries, age 18 or older with known coronary risk factors or CAD, willing to make comprehensive lifestyle changes for one year. This protocol was approved by the Department of Clinical Investigation/Human Use Committee of the Walter Reed Army Medical Center (Washington, DC) and Institutional Review Board at the Uniformed Services University for the Health Sciences (Bethesda, Maryland).

The Coronary Artery Disease Reversal study has been previously described.[7,14] Briefly, subjects participated in a 5-day residential retreat for instruction and initial monitoring of the multicomponent lifestyle change intervention that included: ultralow fat diet (≤ 10% total calories as fat, 5–10 mg cholesterol/d, soy and legumes as the protein source, limited nonfat dairy products, 35–50-g of fiber, and ≥ 5 servings of fruit and vegetables daily), aerobic exercise (≥180 min/wk), and stress management (Hatha yoga poses, deep relaxation, meditation, guided imagery for 60 min/d). During the first 3 months, subjects were on-site twice weekly, 4 hours each visit, for supervised exercise and yoga, meals with educational lectures and group support led by a psychologist. During months 3 through 9 on-site visits were decreased to once weekly. After 9 months, the on-site visits were replaced by weekly telephone monitoring by study nurses and subjects were invited, but not required, to continue subject-directed, group support with their entry cohort.

Subjects voluntarily provided written-informed consent before eligibility screening, which included a complete medical history, physical examination, and treadmill testing. Exclusion criteria included: high-risk treadmill test, unstable coronary artery disease/revascularization procedure within 3 months of study entry, symptomatic congestive heart failure with ejection fraction < 35%, inability/unwillingness to fully participate in all study intervention components, or substance abuse, including tobacco, within 3 months of study entry. A total of 714 patients were referred for recruitment between February 2000 and March 2004 from which 200 subjects enrolled and 186 subjects subsequently initiated the lifestyle program in 13 study cohorts. The final cohort completed the one-year study in April 2005. There was a 23% dropout rate after one year in those subjects who enrolled or initiated the lifestyle intervention, with 166 subjects (89%) completing the 3-month milestone and 144 subjects (77%) completing the year long study. The major reasons for discontinuing study participation were dissatisfaction with specific aspects of the study intervention, time constraints, and relocation away from the study site. The CIMT substudy began in April 2000. Of 130 subjects with baseline carotid ultrasonography, 93 completed the study. Both baseline and 1-year CIMT measures were available for 60 subjects because of missing or noninterpretable images.

Data Collection

Carotid B-Mode Ultrasound

Carotid ultrasonography was performed at baseline and 1 year by study nurses and sonographers specifically trained to perform the research study examinations. Images were obtained on a single ultrasound machine (SonoHeart Elite; SonoSite, Bothell, Washington) using a linear array 5 to 10-MHz transducer with standardized image settings, including resolution mode, depth of field, and gain and transmit focus. All sonograms were obtained with subjects in the supine position and head turned toward the contralateral side. Digital images from a diastolic frame of the cineloop recording were electronically stored and transferred to an off-line workstation for later analysis. Each ultrasound scan was performed as an independent study, without knowledge of the earlier CIMT result, and a subject's earlier scan was not used to guide the follow-up examination. A single independent observer, who was blinded to the study phase of image acquisition and trained in the measurement of CIMT, performed the analyses with commercially available software (ProSolv Echo Analyzer; Problem Solving Concepts, Indianapolis, Indiana). CIMT was determined from images of the far wall of the distal common carotid arteries (immediately proximal to the carotid bulb) and reported as the mean value for the bilateral measurement. The near (intimal-luminal interface) and far (medial-adventitial interface) field arterial wall borders were manually traced for measurement of mean CIMT (mm) across a 10-mm arterial segment. The high precision and reliability of the ultrasound method and reproducibility of the CIMT measurements (>0.90 correlation coefficient) have been previously reported.[15]

Laboratory, Body Composition/Fitness, Blood Pressure, and Nutritional Analyses

Variables measured at baseline and 1 year included: blood pressure (BP) by standard auscultatory methods, weight and body mass index (BMI) by a factory-calibrated Tanita Body Composition Analyzer (Model TBF—300A; Tokyo, Japan), % body fat (3-site skin-fold caliper analysis as described by Pollock[16]), fitness (peak metabolic equivalent [MET] level achieved on maximal treadmill exercise test), fasting plasma lipids (total cholesterol, LDL cholesterol [LDL-C], HDL cholesterol [HDL-C], and triglycerides), and high-sensitivity C-Reactive Protein. Total cholesterol, LDL-C, HDL-C, and triglyceride values were directly measured on a COBAS INTEGRA analyzer using reagents from Roche Diagnostics (Indianapolis, IN). C-Reactive Protein (CRP) was measured with a high-sensitivity, commercially available immunoturbidimetric assay that uses monoclonal anti-CRP antibodies (Roche COBAS INTEGRA, Basel, Switzerland). Nutrient composition was determined at baseline and at the visit closest to week 52 with 3-day food records that were analyzed with Nutritionist V software (Version 2.2; First DataBank, San Bruno, California). Medications were assessed at baseline and any changes in medications or dosage were queried on a weekly basis by study nurses.

Adherence

Intervention adherence was determined from daily personal adherence logs. Overall adherence was calculated as the arithmetic average of adherence to each of the intervention components. Diet adherence was capped at 100% and calculated with a scoring system on the basis of essential elements of the vegan dietary pattern (avoidance of meat/poultry/fish and added oils, intake of specified servings of whole grains, fruits, vegetables, legumes, and soy protein). Exercise (weekly minutes of structured exercise activity) and stress management (combined weekly minutes of previously described techniques) adherence were not capped at 100%, but calculated as the percentage of goal achieved, which was 180 minutes and 420 minutes, respectively. Logs from weeks 39 to 52 were used to calculate adherence at 1 year.

Statistical Methods and Analysis

The 1-year change in mean CIMT across the substudy population (1-year–Baseline CIMT) was evaluated with a paired *t* test. An investigator developed Heart Health Index (HHI) score (range, 0–5), modeled after several healthy lifestyle index scores[17,18] was calculated for 1-year completer subjects ($n = 144$) with 1 point given for each criteria met in the 5-component index: Fiber intake > 25g/d; exercise ≥ 150 min/wk; LDL-C < 100 mg/dL; BMI < 25 kg/m^2; BP < 140/90 mm Hg. The effect of the number of CV health measures achieved (HHI Score) on CIMT changes over 1 year was determined using one-way analysis of variance for paired comparisons.

Analyses on other continuous outcome variables between baseline and 1 year also utilized a one-way analysis of variance for paired or independent comparisons, as appropriate. Fisher's Exact test was used for analyses of all categorical variables. The Wilcoxon Signed Rank test was used for the variables not normally distributed (CRP and triglycerides). Sample size varied slightly across some of these analyses because of missing data on some variables. Values are reported as mean ± SD, except where indicated.

Power estimation was not done for this specific sub analysis. A 2-sided probability value of ≤0.05 was considered as statistically significant. Statistical analyses were performed using SAS statistical software (Version 8.2; SAS Institute, Cary, North Carolina) and SPSS statistical software (Version 14.0; SPSS, Chicago, Ilinois).

RESULTS

Subjects were predominantly older Caucasian men with chronic CAD and/or CVD risk factors (Table I). The CIMT substudy population was comparable with all study completers except that they were slightly younger. Study completers having baseline ultrasonography (n = 93) and the CIMT substudy completers also did not differ in their baseline CIMT values. Overall study intervention adherence was approximately 90%. Exercise and dietary adherence were well-maintained at 1 year (≥90% study goals), whereas the time reported for stress management was 57% of goal.

Medication use was relatively stable throughout the study. At baseline, subjects with hypertension (HTN) were taking antihypertensive medications. Proportion of medications use (baseline to 1 year) was β-blocker (70–73%), angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (63–70%). Despite an increase in the proportion of types of medications used at study completion, dose comparisons showed that 50% of subjects with HTN experienced no change, 23% decreased medication dosage, and 28% had an increase in medication use. From baseline to 1 year, medication use in persons with diabetes increased from 80 to 90%. No change or a decrease in glucose-lowering medications were seen in 70% of the diabetic subjects; however, the proportion of combination therapy (insulin plus oral agent use) decreased from 10 to 0%, whereas oral agent use increased from 70 to 90% of diabetic subjects. Cholesterol-lowering medication use in subjects with dyslipidemia increased from 86 to 88% at 1 year; however, 59% reported no change, 15% a decrease, and 26% an increase in their cholesterol-lowering therapy. Statin therapy increased slightly (76–79%) as well the use of niacin (14–17%), and fish oil (12–16%).

Over one year there was significant improvement in body composition, fitness, BP, and lipid profiles (Table II). BMI decreased by nearly 7% and there was a 10% decrease in percent body fat. Fitness improved 25% as measured by an increase of 2.3 METs. BP was well-controlled at baseline but further improvements in systolic (−4.0%) and diastolic (−3.6%) were demonstrated. On a background of relatively stable lipid medications, a further reduction of 6% occurred in total cholesterol and 8% reductions in both LDL-C and triglycerides. The inflammatory marker, CRP, also improved significantly, decreasing by 9%. The estimated energy consumption and protein intake remained stable, whereas other nutrient composition changed significantly. Subjects reported a baseline low-fat diet and maintained dietary fat intake at 10% of total calories with adherence to a vegan diet. The proportion of dietary carbohydrate increased substantially, largely through a 136% increase in fiber intake.

At study entry, <55% of study subjects met HHI score components, except for BP (Figure 1). Thirty percent of subjects exercised for at least 150 minutes per week, nearly 77% were overweight, and only 35% reported a dietary fiber intake >25 g. Almost 55% had an LDL-C under 100 mg/dL. After one year of study participation, subjects significantly improved achievement of individual HHI score components. At least 62% of subjects reached the individual index criteria for fiber intake, exercise, BP, and LDL-C. Although subjects demonstrated significant weight loss, the proportion of subjects able to achieve BMI in the normal range was less

TABLE I. Demographics, Baseline Characteristics and Study Intervention Adherence: CIMT vs. All 1-Year Completer Subjects

	CIMT Subjects (n = 60)	All 1-Year Completers (n = 144)	p Value
Age (Years)	58.6 ± 9.5	60.6 ± 9.7	0.03
Female (%)	21.7	28.5	0.14
Caucasian (%)	81.7	84.0	0.88
BMI (kg/m^2)	30.3 ± 5.7	29.8 ± 5.8	0.39
CAD (%)	60.0	68.1	0.10
HTN (%)	65.0	67.4	0.72
Diabetes (%)	15.0	18.1	0.51
Hyperlipidemia (%)	96.7	95.8	1.00
Baseline CIMT (mm)	0.731 ± 0.151	0.729 ± 0.159[a]	0.94
Adherence (Overall, %)[b]	91.9 ± 20.6	92.4 ± 22.0	0.88
Diet (% Specified Pattern)[b]	90.5 ± 9.8	89.5 ± 14.1	0.61
Exercise (% Time ≥ 150 min/wk)	95.4 ± 35.5	94.8 ± 44.6	0.93
Yoga (% Time ≥ 420 min/wk)	50.7 ± 27.1	57.9 ± 37.0	0.18

[a] n = 93 for completers with baseline CIMT. [b] n = 143 for all 1-Year completers due to missing dietary records.

TABLE II. Serology, Body Composition/Fitness, BP, and Nutrition in CIMT Population

	Baseline	1 Year	Change	*p* Value
Body Composition and Fitness (*n* = 60)				
Weight (lbs)	204.5 ± 45.1	190.6 ± 45.4	−14.0 ± 18.9	<0.0001
BMI (kg/m^2)	30.3 ± 5.7	28.2 ± 5.7	−2.1 ± 2.8	<0.0001
% Body Fat	27.3 ± 7.5	24.8 ± 7.9	−2.6 ± 3.3	<0.0001
MET Level	9.7 ± 2.7	12.0 ± 3.6	2.3 ± 2.1	<0.0001
BP (*n* = 60)				
Systolic (mm Hg)	125.6 ± 14.5	120.4 ± 14.6	−5.1 ± 14.4	0.007
Diastolic (mm Hg)	73.2 ± 10.2	70.1 ± 9.5	−3.2 ± 9.8	0.015
Serology (*n* = 60)				
Total Cholesterol (mg/dL)	170.9 ± 40.0	159.7 ± 37.9	−11.2 ± 25.2	0.001
LDL-C (mg/dL)	98.2 ± 29.0	89.2 ± 27.2	−9.0 ± 21.8	0.002
HDL-C (mg/dL)	48.3 ± 12.9	47.3 ± 11.8	−1.0 ± 6.8	0.256
Triglycerides (mg/dL)	147.0 ± 90.5	143.1 ± 67.8	−3.8 ± 67.8	0.506
C-Reactive Protein (mg/L)	3.4 ± 4.2	2.3 ± 2.5	−1.1 ± 3.4	0.003
Nutritional Values (*n* = 44)				
Total Kcal	1919.6 ± 488.2	1780.6 ± 388.4	−139.0 ± 554.9	0.104
% Fat	25.2 ± 9.6	10.1 ± 2.6	−15.2 ± 9.7	<0.0001
% Carbohydrate	55.5 ± 12.0	71.9 ± 5.2	16.4 ± 11.6	<0.0001
% Protein	17.1 ± 3.8	15.9 ± 2.6	−1.2 ± 3.8	0.043
Fiber (g/d)	26.1 ± 11.9	51.2 ± 15.0	25.1 ± 17.2	<0.0001

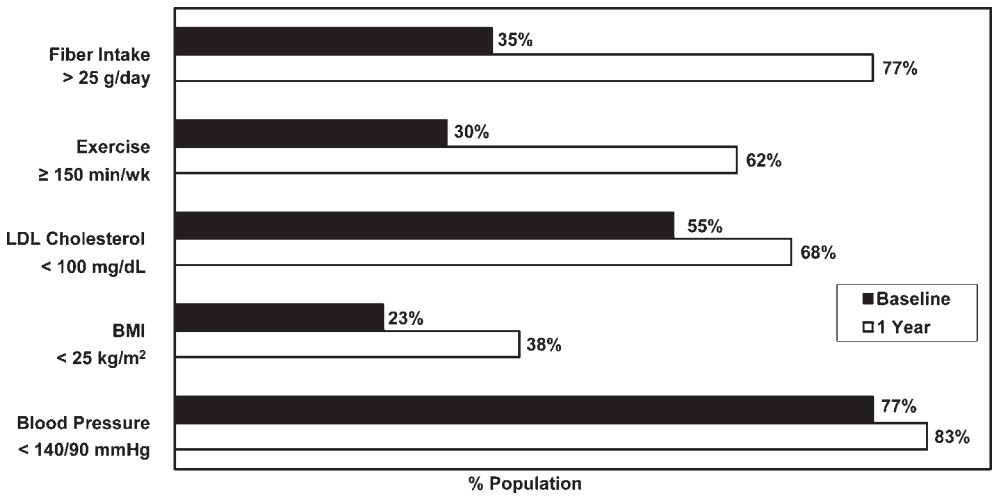

FIGURE 1. Individual heart health characteristics at baseline and 1 year. Changes in distribution for fiber, exercise and BMI are statistically significant at $p < 0.004$ vs. baseline.

dramatic, with 38% of subjects meeting this goal compared with 23% at study entry. The HHI score at study entry was 2.2 ± 1.4 and improved to 3.3 ± 1.2 by 1 year compared with baseline ($p < 0.0001$). An HHI score ≥ 3 was found in only 38% of subjects at baseline, whereas at 1 year 75% achieved this category ($p < 0.0001$). Only 7% of the study population met all 5 HHI criteria at baseline, but improved to 13% at 1 year.

Given that CIMT values exceeding the 75th percentile for age and gender are generally considered abnormal[19,20]; 68% of the population (41/60) was abnormal at baseline with a numerical increase to 77% at 1 year ($p = 0.20$). No difference was detected in mean change in CIMT (−0.011 ± 0.118 mm) in this population, though there is wide variability seen in the individual CIMT values. Decreases, increases, and no change in CIMT were seen in 57%, 43%, and 0% of the population, respectively.

The number of achieved HHI measures correlated with 1-year CIMT change. In subjects with an HHI score ≥ 3 ($n = 45$) CIMT decreased (−0.025 ± 0.120 mm) compared to an increase (0.033 ± 0.102 mm) in HHI score < 3 ($n = 15$) subjects (95% Class Interval = −0.04 to 0.012; $p = 0.10$) (Figure 2). There was a trend for lower baseline CIMT in the HHI < 3 group (0.682 ± 0.159 mm vs. 0.747 ± 0.147 mm; $p = 0.16$). No differences were detected in within group comparisons of CIMT change; however, there was a trend toward atherosclerotic progression in the HHI <3 group

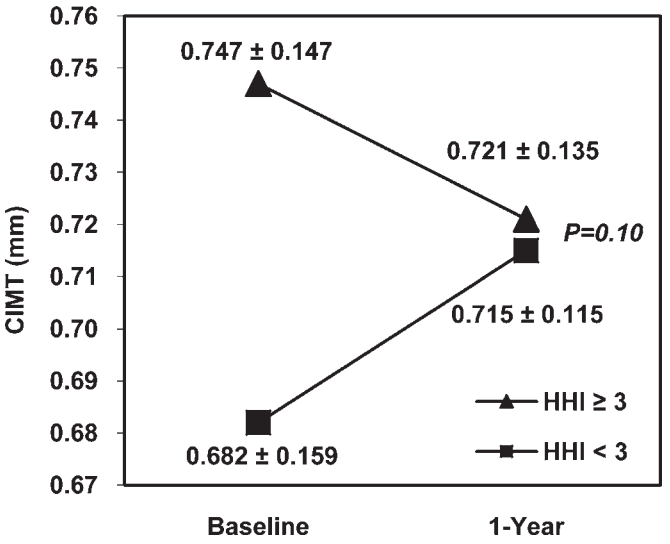

FIGURE 2. Baseline to 1-year CIMT change: comparison between HHI ≥ 3 and HHI < 3 subjects.

TABLE III. Individual HHI Measures and CRP: Comparison Between HHI ≥ 3 and HHI < 3 Subjects (% Change from Baseline to 1 Year)

	HHI < 3 Group (n = 15)	HHI ≥ 3 Group (n = 45)	Between Group, p Values
BMI	−2.6 ± 13.2	−8.2 ± 5.9*	0.028
Exercise	110.7 ± 158.7	67.2 ± 99.9*	0.297
BP			
Systolic	1.0 ± 10.6	−5.7 ± 10.3*	0.036
Diastolic	1.2 ± 14.6	−5.3 ± 12.1*	0.093
LDL-C	2.1 ± 25.9	−11.0 ± 14.8*	0.019
Dietary Fiber Intake	55.9 ± 45.8	142.1 ± 127.3*	0.254
C-Reactive Protein	16.0 ± 70.5	−17.2 ± 39.8*	0.027

*Within group $p < 0.01$.

($p = 0.23$) compared to a regression trend in the HHI ≥ 3 group ($p = 0.16$). Although differences were not found between or within groups for CIMT change, the sub analysis was not specifically powered to rule out significant differences with this number of subjects enrolled.

Of the individual HHI measures (Table III), the most significant differences between HHI ≥ 3 and < 3 subjects were in LDL-C (−11% vs. +2.1%; $p = 0.02$), systolic BP (−6% vs. +1.0%; $p = 0.04$), BMI (−8% vs. −2.6%; $p = 0.03$), and CRP (−17% vs. +16%; $p = 0.03$). Within group comparisons of HHI measures demonstrated no differences from baseline in the HHI < 3 subjects, whereas all measures, except BMI, improved significantly in the HHI ≥ 3 subjects.

DISCUSSION

This study demonstrates that military health care beneficiaries with chronic CAD or CVD risk factors who fully participate in a multicomponent lifestyle intervention program can realize not only substantial improvement in body composition, fitness, BP, lipids, and inflammation, but also an absence of atherosclerosis progression as measured by CIMT, a validated marker of atherosclerosis progression.[21] This finding is likely attributable to the lifestyle intervention as subjects were on relatively stable drug treatment throughout the study, including lipid-lowering therapies. CIMT did not change significantly for the total study population, despite the overall beneficial changes in CVD risk factors. Only the number of commonly recognized CV health goals achieved, correlating with significant reductions in BMI, BP, LDL-C, and CRP, differentiated those subjects with a trend toward CIMT regression vs. progression.

Unlike for pharmacologic therapies[15,22,23], there are no large trials that have evaluated the impact of lifestyle interventions on atherosclerosis progression. The Lifestyle Heart Trial, from which the intervention regimen in our study was adapted, used quantitative coronary angiography to demonstrate a 4.5% coronary stenosis improvement in the experimental group compared with a 5.4% worsening in the control group after 1 year.[8,9] Although our study had no control group and a small substudy group, the magnitude of CIMT change in the group with the most (−2%) compared with least (+7%) CVD risk factor reduction is similar to that study. More recent studies have assessed the impact of various lifestyle changes on CIMT. Using a similar lifestyle change program to ours, Fields et al[24] demonstrated a significant CIMT decrease (−0.15 mm/yr) in 20 intervention subjects, although there was no difference between them and subjects in the comparison control groups. When comparing participants in an Ornish lifestyle program ($n = 46$) to those in a traditional cardiac rehabilitation program ($n = 47$), Aldana et al[25] was also unable to demonstrate a significant reduction in CIMT. A 6-month diet, exercise and behavior modification program in type 2 diabetics significantly reduced CIMT compared with control subjects (−0.04 mm vs. 0.083).[26] Other studies have reported reduction in CIMT progression,[27] but not regression. Weight loss after bariatric surgery was associated with threefold less CIMT progression (0.024 vs. 0.068) compared with obese controls.[28] Reduction of dietary fat intake along with smoking cessation, and BMI decrease of 5 units was associated with a 0.13-mm/yr CIMT reduction in progression.[29] In menopausal women, a dietary and physical activity intervention slowed CIMT progression compared with control subjects (0.008 vs. 0.004 mm/yr), the lower magnitude of effect consistent with a less intense intervention and smaller change in CVD risk factors than seen in our study.[30] CIMT progression was lower in subjects with the greatest vs. least reduction in saturated fat (0.03 vs. 0.10 mm/yr).[31] Some lifestyle intervention studies have not demonstrated any effect on CIMT.[32–35] The small study populations and the magnitude of CVD risk factor change likely explain the variability of CIMT effect reported. Lipid improvement is an important factor, as the extent of CIMT change has been significantly related to LDC-C changes in pharmacologic studies.[36]

There is potential for lifestyle change to have a favorable impact on morbidity and mortality. A recent review suggests

that about a 40% reduction in all-cause mortality might be realized by CAD patients who practice a healthy lifestyle.[37] In The Health Professionals Follow-up Study, men who adopted ≥2 lifestyle practices over 16 years had a 27% lower risk of CAD and 62% of their CV events might have been prevented with the best adherence to recommended lifestyle practices.[18] Individuals achieving four diet and lifestyle factors in the HALE project had a 64% lower rate of CAD death.[38] Large-scale epidemiologic studies have found a significant association between CIMT progression and CV events. The Rotterdam Study, Cardiovascular Health Study and The Atherosclerosis Risk in Communities Study demonstrated 1.3- to 1.7-fold higher risk of myocardial infarction for approximately each 0.2-mm CIMT increase.[10,13,39] Prospective data from the Carotid Atherosclerosis Progression Study confirms these earlier findings across a wide age range.[12] In Carotid Atherosclerosis Progression Study, each 0.16-mm CIMT increase was significantly predictive of a 1.45-fold higher incidence of myocardial infarction, stroke, or death. Thus, the reduction of CIMT among subjects achieving the greatest number of CV health measures in our study supports the potential of lifestyle intervention to reduce future CV morbidity and mortality. Larger clinical trials of lifestyle interventions that assess CIMT as an endpoint are needed to provide convincing evidence in this regard. In the interim, our findings may be utilized to motivate better adherence in lifestyle change programs to maximize their benefit.

Study Limitations

Our study is not a randomized trial and, thus, is subject to the limitations common to all observational studies. The relatively small sample size, along with a self-referred, highly motivated, predominantly male, nonsmoking population increases the referral bias. The relationships analyzed between CV risk factor changes and CIMT progression were performed in a smaller subgroup, thus adding to bias or confounding from unmeasured factors.

CONCLUSIONS

Lifestyle intervention can lead to a delay in atherosclerosis progression, but may depend on the extent of CV health measures achieved. This finding supports an intensive, case-managed program to fully leverage nonpharmacologic approaches for CV risk reduction. Prospective studies are needed to improve understanding of the effects of lifestyle intervention on atherosclerotic progression.

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FEASIBILITY OF INCLUDING LIMITED MINDFULNESS TRAINING IN AN EXISTING THERAPEUTIC LIFESTYLE CHANGE (TLC) PROGRAM

Nancy S. Saum, MS, AHN-BC, Elaine Walizer, MSN, Marina Vernalis, DO, Henry M. Jackson Foundation, Rockville, MD

Background/problem being addressed: Since mindfulness (the simple act of paying attention to what is happening in any moment--without judgment or criticism), encourages us to take greater responsibility for our choices, a potential role for mindfulness in improving diet and exercise lifestyle habits and behaviors has been suggested. However, most often, mindfulness training occurs as a referral to a separate, time-intensive program.

Description: This presentation describes the novel integration of brief mindfulness training into an existing TLC curriculum within a cardiovascular risk reduction program. Abbreviated (10-minute) mindfulness practices (mindful eating, body scan, awareness of breath, mindful movement, walking meditation) were successfully incorporated into 12 weekly 1-hour support groups. Over a 2.5 year period, 142 participants met as 30 cohorts, for a total of 360 support group sessions.

Evaluation: All 142 group members participated in the mindfulness practices. On the post-curriculum evaluations (n=123), the mean participant rating for “meeting personal objectives and expectations” (weight loss and increased physical activity) was 4.6, on a scale of 1(low) to 5(high). Stated benefits of the mindfulness practices included: relaxation, self-compassion, body awareness, increased patience, improved sleep, greater health consciousness, and better management of time and stress. 32% of the respondents also reported incorporating mindfulness practices into their daily lives during the 12 weeks of the TLC program.

Conclusions: While some participants were indeed skeptical, all were willing to learn about mindfulness and participate in the practices. Many participants reported benefit from even brief exposure to mindfulness principles and practice. A formative evaluation of the TLC program is underway to explore the extent of the positive impact of mindfulness and its influence on participant success.

Implications for practice: Abbreviated mindfulness training has the potential to augment the benefits of a TLC program. Further studies are needed to demonstrate its efficacy in health promotion.

Cardiac Rehabilitation Involving Lifestyle Modification Alters Comprehensive Plasma Metabolomic Profiles Defined by LC-FTMS

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Background: Noninvasive coronary artery disease (CAD) management involves risk factor modification through comprehensive lifestyle change. Although lifestyle change is effective in improving traditional CAD risk profiles, little is known about other physiologic responses that influence disease progression. We assessed the hypothesis that metabolomic profiling, which integrates information on dietary, behavioral, and lifestyle factors, can provide important information on CAD risk reduction.
Methods: Patients (n=17) participated in a prospective, nonrandomized, cardiac rehabilitation program designed to stabilize or reverse CAD progression through dietary changes, exercise, stress management, and group support. Nonintervention controls (n=17) were matched to patients based on age, gender, and disease status. Plasma metabolic profiles run in duplicate were generated by liquid chromatography-Fourier transform mass spectrometry (LC-FTMS) and evaluated over 1 year.
Results: Participants showed significant improvement in traditional CAD risk factors at three months and one year. Metabolomic profiling identified 12,859 metabolite features in plasma; 4,432 features were present in more than 90% of the 102 samples analyzed. False discovery rate (FDR, 10%) analysis detected changes in 19 metabolites after 3 months and 7 metabolites after 1 year in participants, but changes in only 1 metabolite at 3 months in controls. At the 1-year examination, 87 differences in metabolite profiles distinguished participants from controls. Metabolites changing significantly in abundance were matched to primarily plant-derived compounds associated with inflammation and platelet aggregation in metabolomics databases (METLIN and Madison Metabolomics Consortium Database). Principal component analysis (PCA) showed clear differences in metabolite abundance during the program and distinct profiles of metabolite change in participants with diagnosed heart disease compared to those with only elevated risk factors.
Discussion: Cardiac rehabilitation involving lifestyle change can effectively alter traditional risk factors and plasma metabolomic profiles, thus reducing risk for cardiac events. In conclusion, metabolomic profiling detects a vast array of diverse metabolites and provides another dimension to understanding complex biological processes involved in cardiac rehabilitation.

Racial Differences in Perceived Stress, Sleep Habits, and Daytime Symptoms

Arn Eliasson MD, Mariam Kashani CRNP, Jacqueline Hoffman MA, Marina Vernalis DO

Introduction: Racial disparities are important to understand in order to design effective programs for evaluation and intervention. We hypothesized that important racial differences exist in subjects enrolling in a heart health program.

Methods: The Integrative Cardiac Health Project (ICHP) is a heart health program that includes goals of improving sleep and stress management. At program entry, participants complete validated questionnaires, specifically the Berlin Questionnaire for sleep apnea, Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), fatigue visual analog scale (FVAS) and the Perceived Stress Scale. Subjects also submit to anthropomorphic measures and a cardiac-relevant lab panel. Differences between whites and blacks were compared using unpaired t-test and Wilcoxon rank sum test (2-tailed) as appropriate.

Results: Of 350 consecutive subjects (mean age 55.1 yrs, 28% men), there were 133 white (38%), 105 black (30%), 90 mixed race/undeclared, 14 Latino, and 8 others. For this analysis, only white and black subjects were considered. White subjects were somewhat older (57.4$\pm$12.6 yr vs 52.1$\pm$12.4, p=0.001) and included more men (47% v 34%, p=0.04). BMI was similar between groups (29.5$\pm$5.1 kg/m^2 vs 30.6$\pm$6.6, p=0.18). White subjects had lower perceived stress (PSS=19.4$\pm$9.6 vs 23.6$\pm$6.8, p<0.001), better sleep quality (PSQI=6.1$\pm$4.1 vs 7.1$\pm$3.9, p=0.05), and less daytime sleepiness (ESS=8.0$\pm$4.9 vs 9.8$\pm$5.0, p=0.01). White subjects tended to have less fatigue (FVAS=3.9$\pm$2.5 vs 4.5$\pm$2.4, p=0.08) and longer sleep duration (20 min longer per night, p=0.07). However, there was no difference in sleep latency (24.4 min vs 23.0, p=0.85) or likelihood for sleep apnea (Berlin positive 44% vs 51%, p=0.40).

Conclusions: There are important differences in levels of perceived stress, sleep quality and daytime sleepiness between white and black subjects in our program. These differences deserve explanation and may be valuable in designing interventions tailored for specific groups.

Sleep Parameters Associated With Hyperinsulinemia Increase CVD Risk

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Mariam Kashani MS, CRNP, Arn Eliasson MD, Marina Vernalis DO

Introduction: Obstructive Sleep Apnea Syndrome (OSAS) is a well-established risk factor for cardiovascular disease (CVD). Multiple mechanisms have been proposed to explain the association between OSAS and CVD. Hyperinsulinemia may play a role in this association.

Objective: We sought to examine the relationship between insulin levels and parameters of sleep.

Methods: Consecutive participants entering a 6-month integrative CVD risk reduction program to improve nutrition, exercise, stress and sleep completed questionnaires including the Epworth Sleepiness Scale (ESS), Fatigue Scale, Pittsburgh Sleep Quality Index (PSQI) and the Berlin Questionnaire for sleep apnea risk. Data collection also included a cardiac-relevant lab panel. Differences between subjects with normal insulin (<20 ug/dL) and abnormal insulin (insulin $\geq$ 20 ug/dL) were analyzed by t-test.

Results: Of 127 consecutive participants entering the program, mean age 51.3 $\pm$ 13.9 years there were 54 men (43%), 65 Caucasian, 49 African-American, 7 Hispanic, 2 Asian and 4 other. Insulin levels in 104 (82%) participants were normal and abnormal in 23(18%). There were no differences in age, gender or race between groups.

	Epworth	Fatigue	PSQI	Berlin	% HgbA1C	CVD Risk Score
Insulin (<20 ug/dL)	9.2+5.5	4.6+2.3	7.3+3.8	49%	5.9+0.9	16.7
Insulin ($\geq$20 ug/dL)	13.5+5.8	5.9+1.9	9.0+4.4	86%	6.8+1.6	23.7
p	0.001	0.02	0.07	0.001	0.001	0.03

Conclusion: Abnormal insulin levels that are associated with unhealthy metabolic and behavioral states place patients at higher risk for CVD. Higher insulin levels associated with increased CVD risk include states of glucose dysregulation, sleepiness, fatigue and high risk for OSAS. We hypothesize that an integrative lifestyle change program can help patients to avoid the resulting adverse consequences of hyperinsulinemia by reducing their insulin levels and potentially providing targets for intervention to reduce CVD risk and to improve parameters of sleep.

The Berlin Questionnaire Identifies a Population with Traits Inhibiting Adherence

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Rationale: As part of a cardiovascular (CV) risk assessment, we screen for sleep apnea using the Berlin Questionnaire. To enhance outcomes of therapies aimed at reducing CV risk it is important to assess for traits that affect adherence to those therapies. We hypothesize that while the Berlin Questionnaire successfully screens for patients with sleep apnea and detects a population at higher risk for CV disease, the survey tool may also identify a population at risk for traits that compromise adherence to therapies for CV risk improvement.

Methods: Consecutive subjects entering our CV disease prevention program completed validated surveys including Berlin Questionnaire for Sleep Apnea and Perceived Stress Scale (PSS). Data collection also included a carotid intima-medial thickness (CIMT) and a CV-relevant lab panel. Differences between subjects scoring positive and those scoring negative for sleep apnea by Berlin Questionnaire were analyzed by t-test and correlations were sought using the Pearson product-moment correlation coefficient.

Results: Of 126 consecutive subjects, demographic data showed a mean age of 51.2±13.9 years, 55 men (44%), and racial diversity (64 Caucasian, 49 African-American, 7 Latino, 2 Asian, and 4 undeclared). Fiftysix subjects (44%) scored a high likelihood of sleep apnea on the Berlin Questionnaire and their CV health assessments demonstrated a higher risk profile:

	Mean CIMT mm	HDL mg/dL	TG mg/dL	HgbA1C %	HOMA	Dx Dep/Anx	PSS
Berlin Neg n=56	0.718±0.144	60.6±18.2	95.0±47.6	5.7±0.4	1.8±0.9	11%	19.3±6.9
Berlin Pos n=70	0.794±0.153	50.5±14.1	127.0±71.6	6.2±1.4	3.4±3.3	28%	23.6±8.6
t-test p	0.005	0.001	0.005	0.01	0.002	0.02	0.003
Pearson r	0.25	-0.30	0.25	0.22	0.31	0.21	0.27

There were no differences between groups for other traditional risk factors such as total cholesterol (p=0.43), LDL cholesterol (p=0.08), lipoprotein (a) (p=0.63), and CRP (p=0.12).

Conclusions: The Berlin Questionnaire does help identify a population of subjects at

greater risk for CV disease as well as traits of anxiety and perceived stress that may diminish the patients' ability to comply with CV risk reduction therapy. An important implication is that sleep apnea therapy should be implemented not only to improve CV risk but to aid the management of anxiety trait and perceived stress trait with the goal of enhancing adherence to behavior change and improving overall quality of life.

Journal Citation: Eliasson A, Kashani M, Bailey K, Vernalis M. The Berlin questionnaire identifies a population with traits inhibiting adherence. Am J Respir Crit Care Med 2011; 183:A1444

Should Subclinical Hypothyroidism Be Treated to Lower Cardiovascular Risk?

Maren Mayhew MS, CRNP, Arn Eliasson MD, Mariam Kashani MS, CRNP, Marina Vernalis DO

Background: Subclinical hypothyroidism (SCH) is diagnosed when TSH is mildly elevated but thyroid hormone levels are normal. Treatment guidelines endorse individualized therapy, offering thyroid replacement for SCH only when symptoms of hypothyroidism are clinically convincing. However, SCH has been associated with increased risk of coronary heart disease (CHD) and while controversial, research has shown that replacement therapy may improve CHD risk factors. In order to inform therapeutic decisions in our cardiovascular disease prevention program (CPP), a program managed by Nurse Practitioners, we sought to evaluate the important health associations of SCH in subjects entering our CPP.

Methods: Patients entering our CPP through self-referral or referral by a provider are evaluated by a Nurse Practitioner with history and physical examination, anthropometrics, a panel of laboratory tests, and validated questionnaires assessing sleep behaviors and stress levels. Consecutive patients over a two year period were considered in this analysis. Patients with the diagnosis of overt thyroid disease and patients on thyroid replacement therapy were excluded. Using a TSH cutoff of >4.2 uIU/dL, subjects with SCH were compared with patients whose thyroid panel was normal, using unpaired t-tests. Relationships between TSH and other continuous clinical variables were assessed with the Spearman's rank-order correlation.

Results: Of 340 consecutive patients, 51 (15%) were excluded for diagnosed thyroid disease or thyroid replacement medication. The remaining 289 patients (165 women) comprised the study set with 111 Caucasian, 89 African-American, 12 Hispanic, 2 Asian and 75 undeclared. There were 10 patients (3.5%) with SCH (6 women, mean TSH 4.74$\pm$0.41) and 279 patients with normal thyroid studies (158 women, mean TSH 1.78$\pm$0.82). For patients with and without SCH, two sample t-tests showed no differences in BMI, waist circumference, perceived stress levels, or C-reactive protein. Indices of glucose metabolism between groups were not statistically different, including fasting glucose, HbA1c, and HOMA. Compared to normal subjects, patients with SCH showed no differences in sleep habits and symptoms, including sleep latency, sleep duration, habitual snoring, risk for sleep apnea, daytime sleepiness and fatigue. Lipid studies showed no statistical differences in total cholesterol (p=0.55), LDL (p=0.71), HDL (p=0.16), TG (p=0.77), PLA2 (p=0.18) or LPa (p= 0.68). Spearman's rank-order correlation showed a statistically significant inverse correlation between TSH level and LPa (rho= -0.146, p=0.012) and identified a correlation between TSH level and HDL (rho= 0.146, p=0.013). Framingham risk index was not statistically different between patients with SCH and normals (p=0.33).

Conclusion: SCH was not associated with an extensive array of CHD risk factors in our population. Our findings support following the current endocrinology guidelines, offering thyroid replacement for SCH only when symptoms of hypothyroidism are clinically compelling. In our Nurse Practitioner managed CPP, the diagnosis of SCH does not appear to warrant thyroid replacement therapy for cardiovascular benefit but should be carefully considered for each patient's circumstances.

Citation: Mayhew M, Eliasson A, Kashani M, Vernalis M. Should subclinical hypothyroidism be treated to lower cardiovascular risk? Conference of the American College of Nurse Practitioners, Tampa, FL, 20-24 Oct 2010, poster presentation

The Need for Cardiovascular Prevention in Young Military Service Members

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Background: Recent data suggest worrisome trends in the prevalence of risk factors for atherosclerosis in Active Duty (AD) soldiers.

Objective: We sought to examine CV risk in a group of young AD members.

Methods: 14 consecutive AD soldiers completed Carotid Intima-Medial Thickness measurement (CIMT--a measure of atherosclerosis), IPAQ (international physical activity questionnaire), BMI, and labs. Differences between subjects with normal and abnormal CIMT (>75% for gender and age) were analyzed by t-test.

Results: Of 14 participants (9 men), average age 27.7 years, 5 had abnormal CIMT. These five soldiers exercised less (524$\pm$183 MET-min/week versus 1577$\pm$1253, p=0.10), showed more snoring/OSA (60% versus 11%, p=0.05), weighed more (BMI=32.4$\pm$5.6 kg/m^2 versus 28.8$\pm$4.0, p=0.18), had dyslipidemia (100% versus 33%, p=0.01), lower HDL (43.2$\pm$11.8 mg/dL versus 55.7$\pm$11.4, p=0.08), and lower vitamin D (12.5$\pm$4.7 pg/mL versus 20.0$\pm$7.9, p=0.08).

Conclusion: In this cohort of young soldiers, subclinical atherosclerosis was prevalent. Reversible risk factors were identified with easily obtained and inexpensive assessment tools. Our experience supports earlier assessment and prevention to conserve the Fighting Force.

Citation: Modlin R, Kashani M, Eliasson A, Bailey K, Vernalis M. The need for cardiovascular prevention in young military service members. Conference of the American College of Physicians—Army Chapter, Bethesda, MD, 18 Nov 2010, poster presentation

The Need for Cardiovascular Prevention in Young Military Service Members

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Citation: Kashani M, Eliasson A, Vernalis M. The need for cardiovascular prevention in young military service members. Force Health Protection Conference, Phoenix, AZ, 10 Aug 2010, poster presentation

Reducing Perceived Stress Improves Sleep Quality—A Longitudinal Outcomes Study

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Introduction: Anecdotal experience suggests that stress is a major impediment to sleep, eroding overall sleep quality. Clinical programs universally endorse interventions for stress reduction to improve sleep, but there are few reports validating this therapeutic approach. To examine the relationship between stress reduction and sleep improvement, we measured changes in perceived stress and its correlation with sleep quality in a longitudinal outcomes study.
Methods: The Integrative Cardiac Health Project (ICHP) is a heart health program with goals of improving diet, exercise, sleep and stress. At program entry and at graduation, participants were assessed with the Perceived Stress Scale (PSS14) and the Pittsburgh Sleep Quality Index (PSQI) which includes sleep duration along with sleep latency, sleep fragmentation, perceived restfulness, daytime functioning, nocturnal behaviors, and use of sleep aids. Subjects were divided into groups that improved PSS score and those that did not. Differences between groups were compared using unpaired t-test.
Results: 66 consecutive graduates (mean age 59.6+11.6, 28 men) reduced their PSS 3.1$\pm$5.8 points and improved their PSQI 1.2$\pm$2.9 points. Fifty subjects were able to reduce their PSS by a mean of 5.5$\pm$4.5 points accompanied by improvements in PSQI (1.9$\pm$3.0 points), Lp-PLA2 (41.6$\pm$53.8 mg/dL), glucose (2.0$\pm$9.1 mg/dL), insulin (2.2$\pm$7.0 ug/dL) and HOMA (0.04$\pm$1.69). The other 16 subjects showed increases in PSS of 4.3$\pm$2.0, $p<0.001$ accompanied by worsening PSQI (0.27$\pm$2.49, $p=0.02$), Lp-PLA2 (21.7$\pm$65.5, $p=0.02$), glucose (2.8$\pm$11.0, $p=0.08$), insulin (1.4$\pm$6.1, $p=0.07$) and HOMA (0.49$\pm$1.51, $p=0.04$).
Conclusions: Reductions in perceived stress correlate significantly with improvements in sleep quality. Improvements in perceived stress and sleep quality are accompanied by improvements in cardiovascular risk markers including glucose metabolism and lipids. Our findings underscore the importance and value of utilizing stress management techniques as a teachable sleep improvement intervention.

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COMPREHENSIVE, EARLY ASSESSMENT IS CRITICAL FOR CARDIOVASCULAR PREVENTION

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Background: During the Korean and Viet Nam conflicts, autopsy findings demonstrated the early development of atherosclerosis in Active Duty (AD) soldiers. Given that cardiovascular (CV) risk factors are on the rise in the US military population, there is a clear need to identify and change reversible risk factors and health behaviors in soldiers long before clinical symptoms present. Prevalence patterns for co-existing risk factors may inform efforts for prevention of CV disease.

Objective: We assessed multiple CV risk factors and health behaviors in a group of young soldiers with hypertension utilizing laboratory studies, anthropometrics, validated questionnaires and sophisticated actigraphic devices to measure activity levels and sleep.

Methods: 12 consecutive AD soldiers entering the Integrative Cardiac Health Project at Walter Reed had lipid and glucose metabolism studies done along with measurement of body mass index (BMI), waist circumference (WC) and % body fat. The soldiers also completed Berlin Questionnaire for sleep apnea, and wore Sensewear actigraphic armbands continuously for up to 5 days. Soldiers with high systolic ($\geq$120 mm Hg) or diastolic ($\geq$80 mm Hg) blood pressure were compared with soldiers who had normal blood pressure using the Student's t-test or chi-square test as appropriate.

Results: Of 12 participants (7 men, average age 27.8 years, 8 Caucasian, 3 African-American, 1 other), 7 had systolic hypertension or diastolic hypertension (6 had both). These hypertensive soldiers were older (32 vs 22 years, p=0.02) and had multiple risk factors for metabolic syndrome: Dyslipidemia (total cholesterol 198 vs 153 mg/dL, p=0.02; LDL cholesterol 122 vs 83 mg/dL, p=0.03); Obesity (BMI 31.9 vs 27.8 kg/m^2, p=0.19, WC 105 vs 85 cm, p=0.01, and % body fat 37 vs 29%, p=0.26); and Glucose Dysregulation (insulin 26 vs 9 uIU/ml, p=0.26; HOMA 5.8 vs 1.7, p=0.27). The hypertensive soldiers also had higher prevalence of sleep apnea (43 vs 0%, p=0.11), and were at markedly higher risk for CV disease (ICHP CV Risk Score 10.3=moderate risk vs 4.8 points=low risk, p=0.02). There were no significant differences in sleep time but soldiers with normal blood pressure exercised ½ hour more each day compared to hypertensive soldiers.

Conclusion: The findings demonstrate clustering of multiple risk factors for CV disease in young soldiers emphasizing the need for comprehensive early assessment for CV disease prevention. In view of the multiple co-morbid risk factors, the use of an integrative intervention strategy may be highly effective.

Citation: Modlin R, Kashani M, Eliasson A, Vernalis M. Comprehensive early assessment is critical for cardiovascular prevention. Conference of the American College of Physicians—Army Chapter, Bethesda, MD, 18 Nov 2010, podium presentation

Novel Tool Improves CV Risk Stratification and Guides Therapy

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Marina Vernalis DO

Background: Accurate risk assessment is of critical importance to any cardiovascular (CV) disease prevention program. Risk stratification tools enable providers to implement appropriate therapies.

Objective: We sought to compare the performance of the Framingham Risk Score (FRS) with a CV Score previously validated by the Integrative Cardiac Health Project (ICHP) in a cohort of subjects with known subclinical atherosclerotic disease by abnormal carotid intima-media thickness (CIMT) measurement.

Methods: Consecutive subjects (n=93) identified with subclinical atherosclerosis by abnormal CIMT ($\geq 75^{th}$ percentile by age/gender) were enrolled in a 6-month CV risk reduction program. Subjects were assessed for past medical history, family history of CV events, anthropometrics and a cardiac-relevant lab panel. FRS and ICHP CV Risk Score were calculated for each individual and were compared. The ICHP CV Risk Score incorporates additional factors such as family history of CV events as well as novel risk factors. All scores were categorized as low, medium and high for CV risk.

Results: In 93 consecutive subjects, mean age was 53.1$\pm$11.13 yrs, 59% women, 47% African-American, 46% Caucasian, 3% Latina, 1% other. Diagnosis of diabetes was present in 13% of subjects. Means: BMI=31.2$\pm$5.3 kg/m^2 , WC=100.2$\pm$13.6 cm, fasting glc=99.1$\pm$35.9 mg/dL, insulin 15.6$\pm$14.4 ug/dL, Tchol=194.9$\pm$42.3 mg/dL, LDL 114.5$\pm$34.0 mg/dL, HDL 56.2$\pm$18.5 mg/dL, TG 117.3$\pm$66.3 mg/dL, Lp(a)=86.5$\pm$92.3 mg/dL, CRP 0.4$\pm$0.6 mg/dL.

By FRS, 12 (14%) subjects scored high risk, 11 (12%) scored medium and 70 (75%) scored low risk. By ICHP CV Risk Score, 4 (36%) of the FRS medium upscored to high risk and 47 (67%) of the FRS low upscored to medium risk. In total, 63% upscored to an appropriately higher risk category by using the ICHP CV Risk Score.

Conclusion: In a population with documented subclinical atherosclerosis and unremarkable conventional risk factor profiles, the ICHP CV Risk Score appeared to be more sensitive in identifying subjects at risk. The ICHP CV Risk Score may be a more discerning tool to guide risk reduction therapy in a prevention program.

INTEGRATIVE CARDIAC HEALTH PROJECT RISK SCORE IMPROVES CARDIOVASCULAR RISK ASSESSMENT IN WOMEN WITH SUBCLINICAL ATHEROSCLEROSIS

Elaine Walizer, MSN, Mariam Kashani, CRNP, Arn Eliasson, MD, Marina Vernalis, DO, Henry M. Jackson Foundation, Rockville, MD

Background: The Framingham Risk Score (FRS) substantially underestimates lifetime risk of cardiovascular disease (CVD), especially in women, when only a 10-year risk model is used. The Integrative Cardiac Health Project (ICHP) Risk Score, which incorporates family history and novel risk factors such as BMI, waist circumference, diastolic BP, LDL-cholesterol, triglycerides, and hsCRP, has been previously validated with carotid intima-media thickness (CIMT), a surrogate marker for atherosclerosis, in middle-aged men where an increase in ICHP Risk Score was associated with increasing CIMT 0.3%.

Objective: To hypothesize that the ICHP Risk Score may improve CVD risk identification in women, we compared risk prediction using FRS and ICHP Risk Score in women with abnormal CIMT.

Methods: 113 non-diabetic female military healthcare beneficiaries underwent clinical and serologic risk factor screening in a study clinic. All had at least 2 CVD risk factors and subclinical atherosclerosis by CIMT (>75th percentile by age/gender). FRS and ICHP Risk Score were calculated and compared.

Results: Of these middle-aged (mean age=54; range 26 to 81), predominately black (50%) women, 4% smoked, 47% were hypertensive and 81% were dyslipidemic including 27% with low HDL; 33% with LDL>130 mg/dL and 18% with triglycerides ≥ 150 mg/dL. Family history of CVD was positive in 65% and 50% had hsCRP ≥ 0.3 mg/dL. Subjects were obese (mean BMI=32; mean waist circumference=100 cm). All subjects were identified as low risk by FRS. When the ICHP Risk Score was applied, 60% shifted from a low to medium risk classification for CVD ($p<0.0001$).

Conclusions: The ICHP Risk Score dramatically improves CVD risk classification in this cohort of women with diagnosed subclinical atherosclerosis.

Implications: These findings emphasize the need for improved CVD risk identification in women. Family history and other novel risk factors add predictive value to current risk models and identify potential therapeutic targets.

Disclaimer: “The views expressed in this abstract are those of the author and do not reflect the official policy of the Department of the Army, Department of Defense, or U.S. Government.”

Journal Citation: Walizer E, Kashani M, Eliasson A, Vernalis M. Integrative cardiac health project risk score improves cardiovascular risk assessment in women with subclinical atherosclerosis. J Cardiovasc Nurs 2011; 26:265

INTEGRATIVE CARDIAC HEALTH PROJECT RISK SCORE IMPROVES CARDIOVASCULAR RISK ASSESSMENT IN WOMEN WITH SUBCLINICAL ATHEROSCLEROSIS

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Background: The Framingham Risk Score (FRS) substantially underestimates lifetime risk of cardiovascular (CV) disease, especially in women, when only a 10-year risk model is used. The Integrative Cardiac Health Project (ICHP) CV Risk Score, which incorporates family history and novel risk factors such as BMI, waist circumference, diastolic BP, LDL-cholesterol, triglycerides, and hsCRP, has shown enhanced predictive performance in middle-aged men.

Objective: To examine our hypothesis that the ICHP Risk Score may improve CV disease risk identification in women, we compared risk prediction using FRS and ICHP Risk Score in a cohort of women with abnormal carotid intima-media thickness (CIMT).

Methods: 128 women underwent clinical and serologic risk factor screening for entry into a lifestyle change intervention study. All had at least 2 CV disease risk factors and subclinical atherosclerosis by CIMT (>75th percentile by age/gender). For this analysis 15 women with diabetes were excluded. FRS and ICHP Risk Score were calculated and compared.

Results: Of 113 non-diabetic (mean age=54, range 26 to 81), predominately black (50%) women, 4% smoked, 47% were hypertensive and 81% were dyslipidemic including 27% with low HDL and 33% with LDL>130 mg/dL. Family history of CV disease was positive in 65%. Subjects were obese (mean BMI=32; mean waist circumference=100 cm). Triglycerides were not elevated (mean=109 mg/dL); 50% had hsCRP $\geq$ 0.3 mg/dL. All subjects were identified as having a low 10-year risk by FRS. When the ICHP Risk Score was applied, 60% shifted from low to medium risk ($p<0.0001$).

Conclusions: The ICHP Risk Score dramatically improves CV disease risk prediction in this cohort of women with subclinical atherosclerosis. These findings emphasize the need for improved CV disease risk identification in women. Family history and other novel risk factors add predictive value to current risk models and identify potential therapeutic targets.

Citation: Modlin R, Walizer E, Kashani M, Vernalis M, Eliasson A. Integrative Cardiac Health Project risk score improves cardiovascular risk assessment in women with subclinical atherosclerosis. Conference of the American College of Physicians—Army Chapter, Bethesda, MD, 19 Nov 2010, podium presentation

Stress Therapy Empowers Prevention (STEP): A Healthy-Lifestyle Program for Breast Cancer Patients

Amy Burke*, Jane Haberkorn, Fran Lechak, Judith Sullivan, James Vizza, Marina N. Vernalis, Darrell L. Ellsworth

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*primary author

Purpose: Breast cancer has a significant emotional, psychological, and social impact, often associated with high levels of stress and sleep deprivation, which promotes unhealthy behaviors causing weight gain, decreased physical fitness, and an increased risk for cardiovascular disease (CVD). Because women with breast cancer often exhibit emotional distress for years following diagnosis, it is important to develop programs that effectively manage stress and promote mental and physical health in breast cancer patients.

Methods: This program is designed to help breast cancer patients lose weight, improve strength and endurance, sleep better, and reduce disease risk factors. Women 18+ years of age with breast disease (or at high risk) are offered a program of lifestyle change, consisting of a Healthy Lifestyle intervention for 3 months with instruction and demonstrations on exercise, nutrition, stress reduction, and mind/body health, followed by a five-year follow-up with additional reinforcement to help integrate healthy behaviors into daily life. Testing is conducted at baseline and after the Healthy Lifestyle intervention, with follow-up examinations every 6 months for 5 years. Information collected includes perceived stress/anxiety, sleep disturbances and psychosocial measurements, carotid ultrasound, traditional CVD risk factors (weight, blood pressure, and body composition), and biochemical assays.

Results: Recruitment has been conducted primarily through newspaper ads and newly designed brochures. Of 43 women who expressed interest in the program, 20 have enrolled. Demographic characteristics of participants are: average age 65, 6% with diagnosed CVD, and 61% with breast cancer. Obstacles to recruitment include out-of-pocket costs to patients, lack of local physician referrals, limited time to devote to participation, and lack of knowledge among patients about the benefits of lifestyle change on quality of life or clinical outcome. Once women make the commitment to participate, satisfaction surveys indicate a high degree of satisfaction with the program.

Conclusions: Proven strategies to reduce risk of recurrence and improve quality of life in breast cancer patients are best implemented as a comprehensive program for lifestyle change, empowering the individual patient to make healthy lifestyle choices. Improving the health and well-being of women with breast disease may have a positive impact on breast disease and cardiovascular outcomes.

Category II - Clinical Care, Treatments, and Processing
E. Complementary and Integrative Care
4. Mind/Body Interactions

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Improvement in Cardiovascular Risk Factors in Breast Cancer Patients Participating in the Stress Therapy Empowers Prevention (STEP) Program

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Introduction: Breast cancer is the most frequently occurring cancer and leading cause of death in women between 20 and 59 years of age in the US. Significant emotional and psychological sequelae, including stress and sleep disturbance, often degrade the quality of life in breast cancer patients. Similar to cardiovascular disease, breast cancer susceptibility is influenced in part by modifiable risk factors, suggesting that a healthy lifestyle program may lead to significant improvements in mental health and quality of life as well as reductions in cancer recurrence and cardiovascular risk.

Methods: Eighteen women with breast disease are participating in a lifestyle program designed to improve quality of life and reduce disease risk factors. The intervention provides instruction and demonstrations on exercise, nutrition, stress reduction, and mind/body health, and includes a five-year follow-up with reinforcement to help integrate healthy behaviors into daily life. Examinations at baseline, after the healthy lifestyle intervention, and at one year, collected information on age, ethnicity, health history, medication use, and diet. Psychosocial surveys assessed depression, perceived stress, general well-being, and sleep quality. Physical exams measured heart rate, blood pressure, weight, and percent body fat. Blood was collected to measure lipids, glucose, glycosylated hemoglobin, and a panel of biochemical variables.

Results: Over the initial 12 week period, participants showed change in the desired direction for many risk factors: body weight (-1.8%; $p<0.01$), diastolic blood pressure (-8.5%; $p<0.05$), and perceived stress (-24.2%; $p<0.05$) decreased significantly, while triglycerides (-14.4%; $p=0.09$), systolic blood pressure (-7.6%; $p=0.08$), depression (-25.3%; $p=0.09$), hostility (-25.6%; $p=0.07$), and sleep quality (+20%; $p=0.05$) showed near significant changes. In patients who have reached the one year time-point (n=9), perceived stress (-32.0%; $p<0.05$) and sleep quality (+30.8%; $p<0.01$) improved significantly, and glycosylated hemoglobin levels decreased (-4.5%; $p=0.09$).

Conclusions: A comprehensive program for lifestyle change that empowers patients to make healthy lifestyle choices can successfully improve quality of life and overall health in as little as three months. As participants continue in the program, we will evaluate the health and morale of these women to determine if lifestyle changes result in improved clinical outcomes over the long-term.

Category II - Clinical Care, Treatments, and Processing
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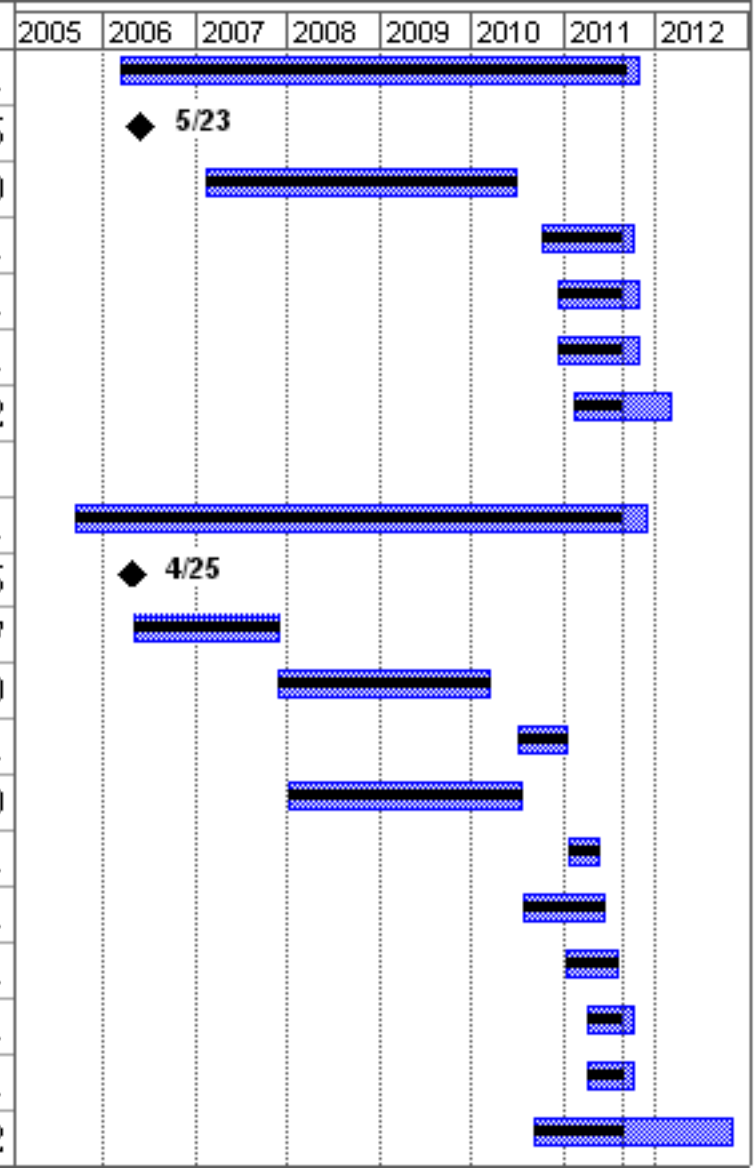
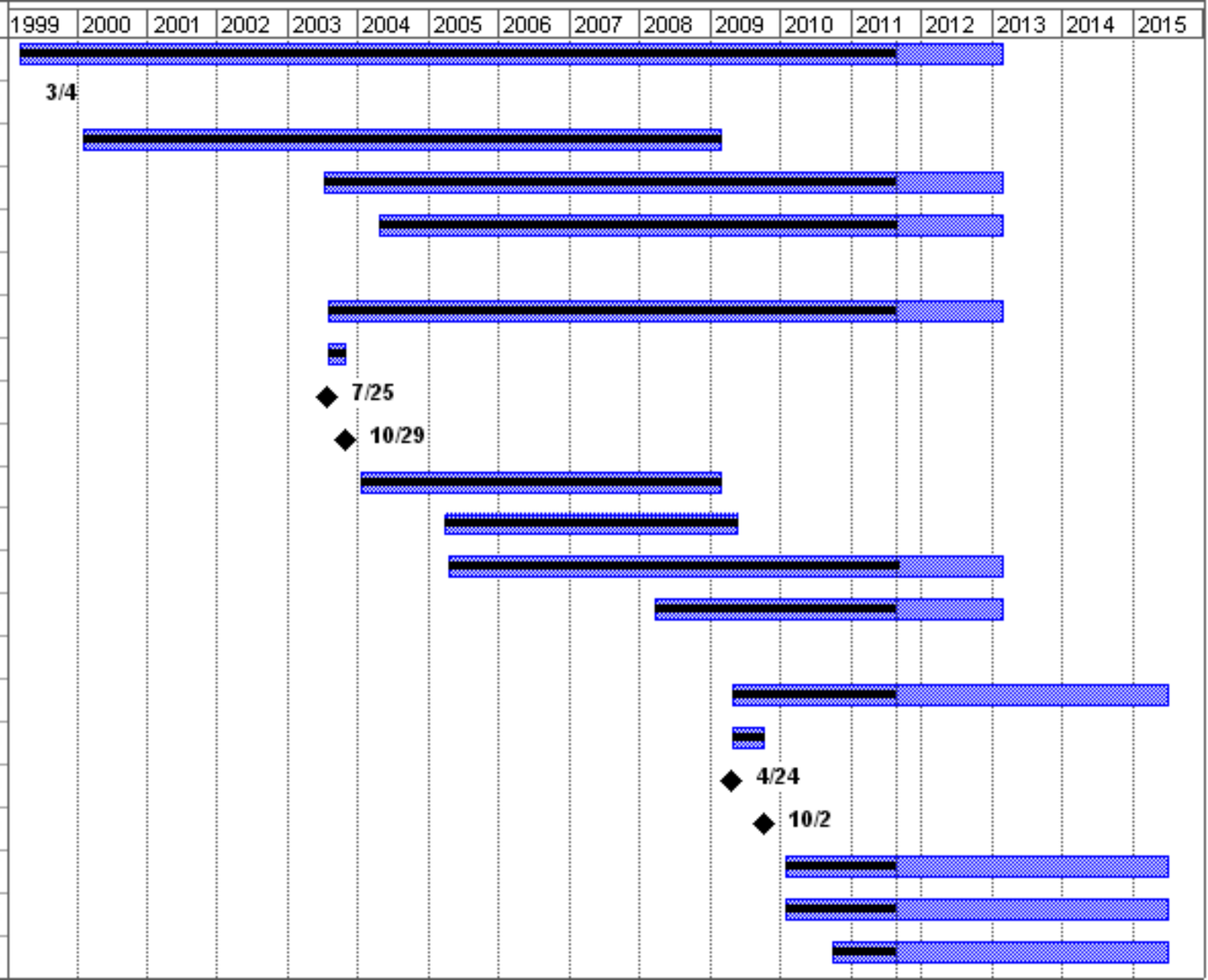
Appendix B
Gantt Charts

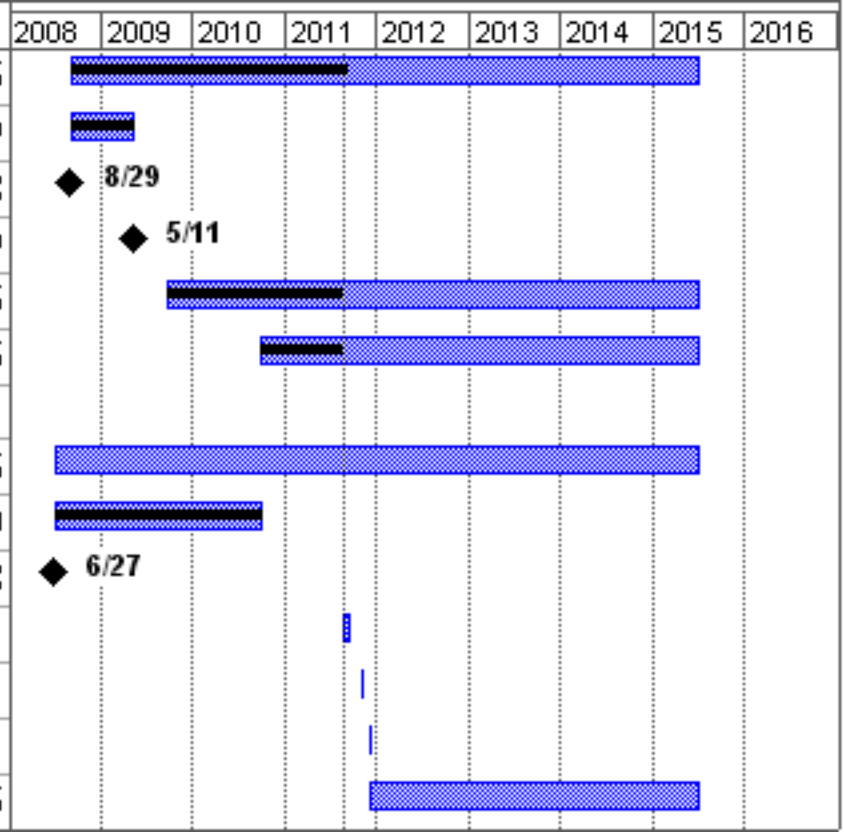
ID	Task Name	Start	Finish
1	Task #3: CADRe Five-Year Follow-up	Wed 3/1/06	Mon 10/31/11
2	IRB protocol approval	Tue 5/23/06	Tue 5/23/06
3	Participant enrollment/Data collection	Fri 2/2/07	Wed 6/30/10
4	Data reconciliation	Fri 10/1/10	Fri 9/30/11
5	Conduct analysis	Wed 12/1/10	Mon 10/31/11
6	Publication plan	Wed 12/1/10	Mon 10/31/11
7	Presentations and manuscripts	Tue 2/1/11	Wed 2/29/12
8			
9	Task #4: BATTLE trial	Thu 9/1/05	Wed 11/30/11
10	IRB protocol approval	Tue 4/25/06	Tue 4/25/06
11	Intervention preparation	Tue 4/25/06	Fri 11/30/07
12	Recruitment/Enrollment/Data	Thu 11/15/07	Wed 3/10/10
13	Addendum submission/approval	Thu 7/1/10	Fri 1/14/11
14	Data collection (Main study)	Tue 1/1/08	Thu 7/15/10
15	Data collection (Addendum)	Tue 1/18/11	Wed 5/18/11
16	Database reconciliation (Main study)	Thu 7/15/10	Wed 6/15/11
17	Data analysis (Main study)	Mon 1/3/11	Fri 7/29/11
18	Quantitative analysis (Addendum)	Fri 4/1/11	Fri 9/30/11
19	Publication plan	Fri 4/1/11	Fri 9/30/11
20	Presentations and manuscripts	Wed 9/1/10	Wed 10/31/12



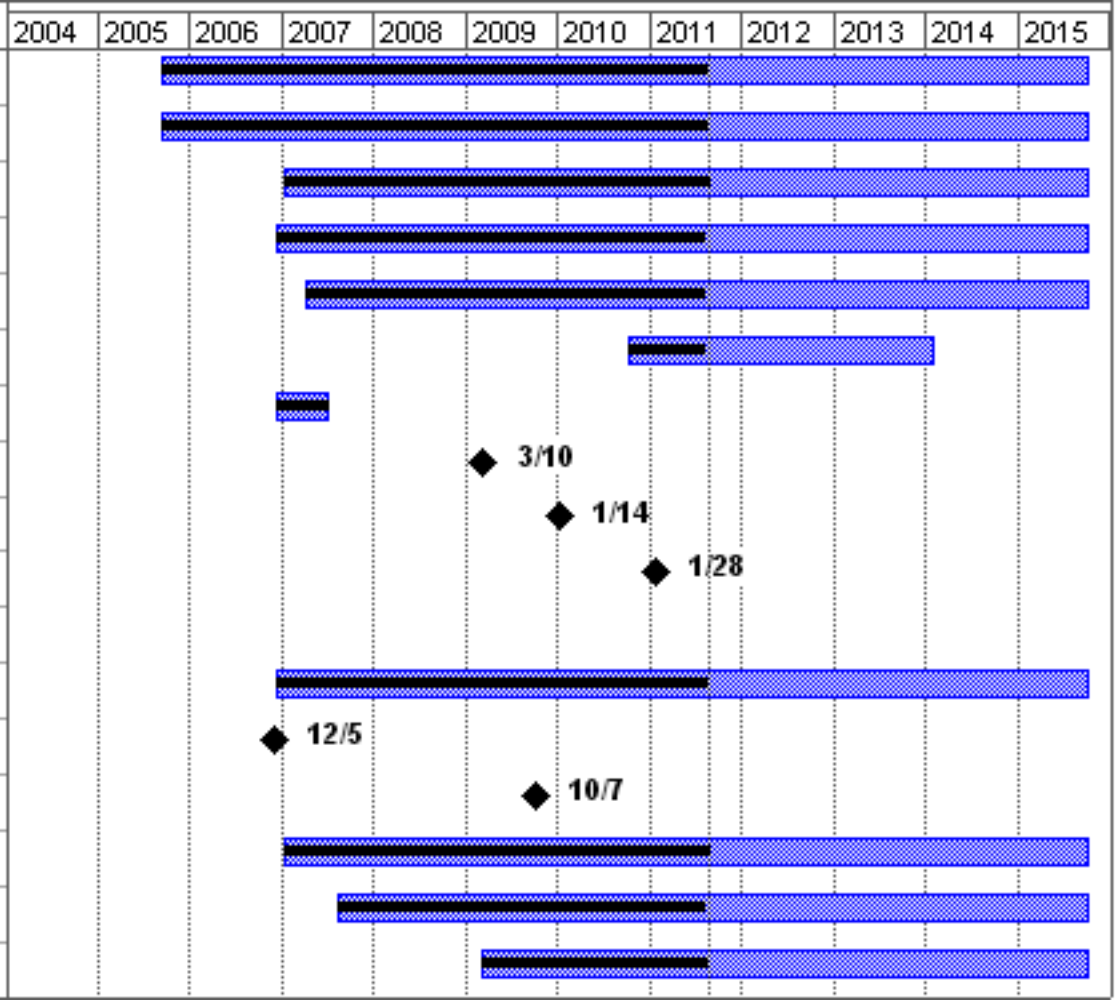
ID	Task Name	Start	Finish
1	Task #5: Ornish Program	Thu 3/4/99	Thu 2/28/13
2	Protocol approved - WMC	Thu 3/4/99	Thu 3/4/99
3	Enroll program participants	Tue 1/25/00	Wed 2/25/09
4	Conduct risk factor analyses	Tue 7/1/03	Thu 2/28/13
5	Presentations & publications	Tue 4/13/04	Thu 2/28/13
6			
7	Task #6: Global Profiling	Fri 7/25/03	Thu 2/28/13
8	IRB protocol development	Fri 7/25/03	Wed 10/29/03
9	Protocol approved - WMC	Fri 7/25/03	Fri 7/25/03
10	Protocol approved - USU	Wed 10/29/03	Wed 10/29/03
11	Enroll program participants	Mon 1/12/04	Wed 2/25/09
12	Enroll control subjects	Mon 3/21/05	Tue 5/19/09
13	Conduct molecular analyses	Mon 4/11/05	Thu 2/28/13
14	Presentations & publications	Tue 3/11/08	Thu 2/28/13
15			
16	Task #7: CRC	Fri 4/24/09	Tue 6/30/15
17	IRB protocol development	Fri 4/24/09	Fri 10/2/09
18	Protocol approved - WMC	Fri 4/24/09	Fri 4/24/09
19	Protocol approved - TATRC	Fri 10/2/09	Fri 10/2/09
20	Enroll program participants	Tue 1/19/10	Tue 6/30/15
21	Enroll control subjects	Tue 1/19/10	Tue 6/30/15
22	Conduct molecular analysis	Wed 9/15/10	Tue 6/30/15



ID	Task Name	Start	Finish
1	Subtask #7a: STEP	Fri 8/29/08	Tue 6/30/15
2	IRB protocol development	Fri 8/29/08	Mon 5/11/09
3	Protocol approved at WMC	Fri 8/29/08	Fri 8/29/08
4	Protocol approved at TATRC	Mon 5/11/09	Mon 5/11/09
5	Enroll program participants	Tue 9/15/09	Tue 6/30/15
6	Conduct molecular analysis	Wed 9/15/10	Tue 6/30/15
7			
8	Task #9: MI in Young Military	Fri 6/27/08	Tue 6/30/15
9	IRB protocol development	Fri 6/27/08	Thu 9/30/10
10	Protocol approved at WMC	Fri 6/27/08	Fri 6/27/08
11	WRAMC protocol development	Wed 8/10/11	Thu 9/15/11
12	Protocol approved at WRNMMC	Mon 10/31/11	Mon 10/31/11
13	Protocol approved at TATRC	Wed 11/30/11	Wed 11/30/11
14	Conduct molecular analysis	Thu 12/1/11	Tue 6/30/15



ID	Task Name	Start	Finish
1	Task #10: Cont. CPP/Prot	Thu 9/1/05	Wed 9/30/15
2	Enrollment/Data collection	Thu 9/1/05	Wed 9/30/15
3	Outcomes analysis	Mon 1/1/07	Wed 9/30/15
4	Target subgroup popns	Fri 12/1/06	Wed 9/30/15
5	Presentations/manuscripts	Mon 4/2/07	Wed 9/30/15
6	Upgrade database	Fri 10/1/10	Fri 1/31/14
7	CORPS Project	Fri 12/1/06	Fri 6/29/07
8	Retro CPP Outcomes Appr	Tue 3/10/09	Tue 3/10/09
9	Retro CPP Outcomes Appr	Thu 1/14/10	Thu 1/14/10
10	Retro CPP Outcomes Appr	Fri 1/28/11	Fri 1/28/11
11			
12	#10.1: Validate CV risk	Tue 12/5/06	Wed 9/30/15
13	IRB protocol approval	Tue 12/5/06	Tue 12/5/06
14	Continuing review approved	Wed 10/7/09	Wed 10/7/09
15	Data collection	Mon 1/1/07	Wed 9/30/15
16	Conduct analysis	Wed 8/1/07	Wed 9/30/15
17	Presentations/manuscripts	Mon 3/2/09	Wed 9/30/15



ID	Task Name	Start	Finish
1	Subtask #10.5: Initiate ZENITH trial	Fri 1/1/10	Fri 10/30/15
2	Protocol development	Fri 1/1/10	Fri 9/30/11
3	Protocol approval	Thu 9/1/11	Fri 9/30/11
4	Recruitment/enrollment/data collection	Mon 10/3/11	Tue 9/30/14
5	Conduct analysis	Thu 10/1/15	Thu 6/30/16
6	Publication plan	Wed 4/1/15	Fri 9/30/16
7	Presentations and manuscripts	Tue 10/1/13	Fri 12/30/16